











October 1, 1988- September 31, 1989

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# Cancer Treatment

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**Intramural Activities**

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# Cancer Treatment

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1988 through September 30, 1989

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SUMMARY REPORT  
ASSOCIATE DIRECTOR FOR CANCER THERAPY EVALUATION  
DIVISION OF CANCER TREATMENT

October 1, 1988 - September 30, 1989

**GENERAL ORGANIZATION**

The Cancer Therapy Evaluation Program (CTEP) is responsible for the administration and coordination of the majority of the extramural clinical trials supported by DCT. These programs include the activities of the Clinical Cooperative Groups, the Phase I and Phase II new agent development contractors, and the holders of investigator-initiated grants (RO1 and PO1) relating to cancer treatment. Certain programs in developmental radiotherapy, such as high LET radiation, are administered in the Radiation Research Program. The Phase I development of biologic response modifiers is handled by the Biological Research Modifiers Program.

The Investigational Drug Branch (IDB) is responsible for sponsoring trials of new investigational drugs and of evaluating them for efficacy and toxicity. It does this by: 1) Coordinating and monitoring the trials of new agents developed by the DCT; 2) Planning with members of the Clinical Investigations Branch (see below overall strategies for new agent studies in specific tumor types; 3) Regulating the distribution of investigational new drugs for which DCT is the sponsor; 4) Maintain close contact and ongoing dialogue with the pharmaceutical industry in an attempt to ensure that new agent development proceeds in a coordinated way.

The Clinical Investigations Branch (CIB) is responsible for development and implementation of disease-oriented treatment strategies across the spectrum of human malignancies. In doing so, it provides management and oversight of the clinical cooperative group program. It manages the oncology portfolios of RO1 and PO1 grants.

The Regulatory Affairs Branch (RAB) monitors the conduct of clinical trials performed in the NCI-supported clinical trials network. It also assures that clinical investigators using experimental agents are in compliance with federal regulations regarding the use of such agents. At the start of the clinical testing of each investigational agent, RAB obtains Investigational New Drug (IND) exemption authorization from the Food and Drug Administration (FDA) and maintains close communication with FDA in all matters relating to experimental drug studies.

The Biometric Research Branch (BRB) provides statistical consultation to the other branches of CTEP, to the extramural and some intramural activities of other programs in DCT, and to the statistical centers of the clinical cooperative groups. It also carries on research in statistical methodology relating to cancer clinical trials.

The Office of the Associate Director (OAD) integrates the efforts of the Branches.

The process of protocol review is administered within the OAD by a central Protocol and Information Office (PIO) which is also the receipt point at NCI for all protocols entered into the PDQ system. The Program Analysis and Management Office (PAMO) has responsibility for the technical management of CTEP's grants and contracts and carries out analyses, as needed, of certain fiscal and administrative issues of particular interest to the program. The OAD is responsible for overall program supervision and budgetary allocation.

#### **ORGANIZATIONAL AND PROFESSIONAL STAFF CHANGES**

Robert Wittes, M.D. resigned as Associate Director to become Senior Vice President for Cancer Research, Bristol-Myers Company, Wallingford, Connecticut.

Michael Friedman, M.D. formerly Chief, Clinical Investigations Branch was appointed Associate Director, CTEP.

Linda Hogan, Head, Protocol and Information Office, CTEP resigned to assume a management position at ELM Services, Rockville, Maryland.

Richard Ungerleider, M.D. formerly Head, Pediatric Section, Clinical Investigations Branch, was appointed Chief, Clinical Investigations Branch.

Stacy Nerenstone, M.D. resigned as Medical Officer, Clinical Investigations Branch to go into private practice in New Haven, Connecticut.

Timothy Chen, Ph.D. was recruited as a Mathematical Statistician in the Biometric Research Branch. He was formerly a Senior Statistician at Alcon Laboratories, Inc. in Fort Worth, Texas.

Edward Korn, Ph.D. was recruited as a Mathematical Statistician in the Biometric Research Branch. He was an Associate Professor in the Department of Biomathematics, UCLA School of Medicine and the Jonsson Comprehensive Cancer Center, Los Angeles, California.

Walter Torri, M.D. Ph.D. will spend a year as a Special Volunteer from Italy in the Biometric Research Branch.

Sylvain Durrleman resigned as an Expert in the Biometric Research Branch to take a position at Hopital Ville Juif, Paris, France.

Michael Hawkins, M.D. was appointed Chief, Investigational Drug Branch. He had been Head, Biologics Evaluation Section, Investigational Drug Branch.

Jason Fisherman, M.D. was recruited as Medical Officer in the Investigational Drug Branch. He was formerly a research fellow at Children's Hospital, Boston, Massachusetts.

Lorraine Cazenave, M.D. transferred from COP where she was a Medical Staff Fellow to become a Medical Officer in the Investigational Drug Branch.

J. Mel Sorensen, M.D. was recruited as a Medical Officer in the Investigational Drug Branch. He was formerly a Senior Clinical Research Fellow at the Mayo Clinic, Rochester, Minnesota.

Gisele Sarosy, M.D. transferred from the Investigational Drug Branch to the Office of the Director, Physicians Data Query Section.

Jean Grem, M.D. transferred from the Investigational Drug Branch to the Medicine Branch, COP.

Brian Leyland-Jones resigned his Expert position in the Investigational Drug Branch to become Professor and Chairman, Department of Oncology, McGill University, Montreal, Canada.

Michael Christian, M.D. formerly a Medical Officer in the Developmental Chemotherapy Section, Investigational Drug Branch was appointed Head, Developmental Chemotherapy Section.

## HIGHLIGHTS IN PROGRAM DEVELOPMENT

### 1. Interactions with the FDA

In attempting to facilitate the access of effective new agents into the clinical armamentarium, greater attention has been devoted to improving communications with the IDB and the Regulatory Affairs Branch and relevant staff of the FDA. Regularly scheduled interactions include monthly bilateral meetings, attendance at respective Protocol Review Meetings by FDA and CTEP Staff and the planning of a joint training program for new faculty. The success of such efforts has been demonstrated by the rapid approval of Group C status for Levamisole + 5FU for Dukes' C colon cancer patients.

### 2. Improved Linkages with the Pharmaceutical Industry

Efforts by the IDB to improve communication with Pharmaceutical firms engaged in the development of anticancer agent development has been increasingly successful. Co-development plans have more often been formulated and information more completely exchanged. Especially noteworthy are the successful efforts in obtaining support from 2 companies for selected complex extramural studies of very high interest with interleukin-2.

### 3. Attention to Reimbursement as an Obstacle to Clinical Research

Increasingly (and anecdotally), third parties have been refusing to support the clinical care costs associated with NCI sponsored clinical investigation. CTEP has sponsored meetings of representatives from the extramural research, pharmaceutical manufacturing and lay communities to examine these complex issues. The resulting consensus statement has been submitted for publication. Subsequent meetings are planned and dialogue continues. In an attempt to quantify the magnitude of this issue a joint effort of ASCO and CTEP is ongoing. Physicians will be surveyed nationwide to more precisely define this problem.

#### 4. Minority Cancer

The disproportionate impact of cancer on racial minorities deserves increased attention. CTEP and DEA have implemented a pilot program to increase accrual of minority patients to Cooperative Group clinical trials. A more ambitious program is being designed by the CIB to address this serious need.

#### 5. Cooperative Group Status

The Cooperative Group system has evolved significantly over the past 5 years. As demonstrated by the chart below, this system is better focused and more efficient. The number of Groups has decreased from 18 to 11 and number of studies from 585 to 490 while the number of patient entries has increased.

##### COOPERATIVE GROUP PROGRAM (1985 VERSUS 1988)

COOPERATIVE GROUP	FY 85 BUDGET TOTAL DOLLARS (THOUSANDS)	FY 88 BUDGET TOTAL DOLLARS (THOUSANDS)
BTCG	886	747
CALGB	3,935	7,526
CCSG	5,160	7,801
ECOG	5,388	8,550
GOG	3,504	2,644
LCSG	1,278	1,401
NCCTG	1,055	1,269
NSABP	3,899	6,297
POG	4,352	5,519
RTOG	3,487	4,000
SWOG	5,020	8,630
GITSG	1,002	
MAOP	603	
NBCP	1,710	
NCOG	1,426	
NPCP	1,415	
POA	793	
SEG	2,600	
TOTAL BUDGET	50,789	58,081
MAJOR GROUPS FUNDED	18	11
ANNUAL ACCRUAL	18,187*	21,122
STUDIES OPEN TO ACCRUAL	586	495

\*INCLUDING SOME NON-THERAPEUTIC STUDY ACCRUAL



## 6. The Cooperative Groups as a Scientific Resource

While the primary charge to the Groups is to perform high quality therapeutics studies, there is ample (and largely unrealized) potential for performing correlative scientific studies. These comparison studies include examining such issues as prognostic factors (egs. oncogenes, ploidy, etc.) drug resistance, pharmacology, and clinical immunology. The creative uses of all NCI support mechanisms are being considered. Additionally, other NCI Divisions are being urged to consider the Groups as a valued resource for performing their studies. Currently, both DCPC and DCBD are exploring ways of increasing their use of Group expertise, patient populations, or data/specimen banks. CTEP is participating in the Diagnosis Decision Network Committee to foster such interactions.

### STAFF PUBLICATIONS:

Avis FP, Ellenberg S, Friedman MA. Surgical Oncology Research: A disappointing status report. *Annals of Surg.* 1988; 207: 262-266.

Brennan M, Kinsella T, Friedman, MA. Pancreatic cancer. In: DeVita VT, Rosenberg S, Hellman S, eds. *Cancer: Principles and Practice of Oncology*, Philadelphia: JB Lippincott 1988, pp. 800-831.

Cheson BD, Lacerna L, Leyland-Jones B, Sarosy G, and Wittes RE. Autologous bone marrow transplantation: Current status and future directions. *Ann Intern Med.* 1989; 110:15-65.

Cohen A, Shank B, Friedman MA. Colorectal cancer. In: DeVita VT, Rosenberg S, Hellman S, eds. *Cancer: Principles and Practice of Oncology*, Philadelphia: JB Lippincott 1988, pp. 895-952.

Dorr A, Bader J, Friedman MA. Locally advanced breast cancer: Current status and future directions. *Internat J Radiat Oncol Biol Phys*, 1989; 16: 775-784.

Dorr FA, Friedman, MA. Clinical trial design for cytotoxics in prostate cancer. In: Coffey DS, Resnik M, Dorr A, Karr JP, eds. *The management of prostate cancer*. New York: Plenum Publishing Corporation, 1988, pp 267-275.

Eisenberger MA, Ellenberg S, Leyland-Jones B, Friedman MA. The application of a two stage design for clinical trials in patients with recurrence head and neck. *Med Pediat Oncol*, 1989; 16:162-168.

Friedman MA. Cancers of the hepatobiliary system and pancreas. In: Wittes RE. *ed Manual of Cancer Therapeutics*. Philadelphia: JB Lippincott, In press.

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Nerenstone SR, Ihde DC, Friedman MA. Clinical trials in primary hepatocellular cancer: Current status and future directions. *Cancer Treat. Revs.* 1988; 15: 1-31.

Roper M, Friedman MA. Oncology. CONTEMPO '89. Journal of the American Medical Association, 1989; 261:2865-2867.

Shoemaker D, Burke G, Dorr A, Friedman M. A regulatory perspective. In: Spilker B Ed. Quality of Life Assessment in Clinical Trials. New York, Raven Press, In press.

Nerenstone S, Friedman MA. Primary liver cancer. Comprehensive Textbook of Oncology, Second Edition, Chapter 88. In press.

## BIOMETRIC RESEARCH BRANCH

### 1. STATISTICAL PLANNING AND REVIEW OF CTEP SPONSORED CLINICAL TRIALS

The Biometric Research Branch collaborates in the development of clinical trials to evaluate new chemotherapeutic and biological agents. The BRB reviews all CTEP sponsored extramural clinical trials to ensure that they are planned, conducted and reported in a sound and efficient manner. BRB staff interact with extramural investigators and cooperative groups to achieve clinical trial designs that are mutually satisfactory to the NCI and to the extramural organization. The BRB also participates in data monitoring committees and in decisions for early termination or expansion of CTEP sponsored clinical trials. Both design and interim monitoring activities often involve extensive simulation studies and data analyses. BRB staff perform interim analyses of contract supported clinical trials and evaluate reports of promising therapeutic regimens for the planning of possible future clinical trials.

The BRB serves as liaison to extramural statistical centers. BRB staff visits centers and organizes national meetings in order to improve statistical and data management procedures.

### 2. PRECLINICAL DRUG DISCOVERY

- a. Methods for the detection of differential cytotoxicity (histologically related or otherwise) have been developed for the in-vitro colorimetric human tumor cell line assay in collaboration with Drs. Ken Paull, Lou Hodes and Robert Shoemaker of the Developmental Therapeutics Program. The cell line assay is designed to test the in-vitro toxicity of potential anti-cancer agents across a broad spectrum of human tumors. The methods of detecting differential cytotoxicity are based on differences in the approximated IC50 values (the dose levels calculated to result in 50% cell growth inhibition), across the cell line panel. An explanatory manuscript is in press. Further work on the detection of differential cytotoxicity is ongoing. We are developing additional methods based directly on the dose response curve of measured cell inhibition levels, rather than on the calculated IC50 values. An extensive interactive computer package of display and analysis tools, based on these methods, is also in development.
- b. A statistical comparison of two different in-vitro assays (MTT vs SRB) was completed with Drs. Paul and Shoemaker to demonstrate the equivalence of the more practical SRB assay with the previously used MTT assay. The analysis involved several different types of comparisons, based either on the calculated IC50 values or based directly on the dose response curve of cell inhibition levels. It was based on data from 243 compounds tested against the cell line panel with both the MTT and SRB assays. It also included analyses of the reproducibility of the 2 assays. A presentation was made at the annual AACR conference and a manuscript is in preparation.

- c. A set of simulations was completed to determine the required number of cell lines per histologic subgroup to enable the in-vitro cell line panel to effectively detect differential cytotoxicity. It was determined that a cell line panel including 10 histologic subgroups should have at least 10 cell lines per subgroup.
- d. In collaboration with Dr. Hodes of the Developmental Therapeutics program, we have attempted to develop more statistically powerful methods for analyzing results of in-vitro screening of AIDS drugs. These methods are alternatives to the usual T/C based analyses. Initial results are promising and a manuscript is in preparation.

### 3. DISCOVERY OF IN-VIVO SYNERGISM

Methods for the design and analysis of in-vivo murine tumor studies of the efficacy and toxicity of drug combinations, based on response surface methodology, have been developed in collaboration with Drs. Grem, Christian and Hawkins. A large number of experiments have been analyzed in order to identify therapeutically synergistic combinations of cytotoxic and biological agents.

### 4. COMPARATIVE STUDIES TO EVALUATE MAGNETIC RESONANCE IMAGING

The BRB has collaborated with the Diagnostic Imaging Branch (Dr. Matti Al-Aish, Acting Chief) of the Radiation Research Program in the conduct of prospective multi-institution evaluations of MRI relative to CT scanning and other modalities in the diagnosis of brain neoplasms, liver metastases, musculoskeletal tumors, cervical myelopathies, lung cancer, uterine neoplasms, and congenital heart disease. The BRB participated in the following ways:

- a. Primary statistician in the design, supervision and analysis of the following protocols:

- Brain neoplasms (Dr. Meredith Weinstein)
- Cervical myelopathies (Dr. Scott Rosenbloom)
- Congenital heart disease (Dr. Charles Higgins)
- Uterine neoplasms (Dr. Hedvig Hricak)

- b. Chairman of the statistical advisory group for the MRI studies:

- Developed the design for the centralized multi-institutional comparative reading of MRI vs. CT in which semiannual 3-day meetings were held to read approximately 1200 images per meeting. The design balanced potentially biasing effects between the two modalities.

- c. Supervision of the data management contract.
- d. Preliminary analyses were prepared for the NIH Consensus Conference on MRI and final analyses are in progress.

## 5. NATIONAL CLINICAL TRIALS OF EARLY OVARIAN CANCER

- a. BRB staff has served as primary statistician for clinical trials of the staging and treatment of early ovarian cancer. Final analyses of the therapeutic questions have been performed and a manuscript has been submitted for publication. The results indicated that adjuvant chemotherapy was not appropriate for patients with very early stage disease (FIGO stages Ia and Ibi). Post-surgical delivery of chromic phosphate (P32) was as effective as chemotherapy for those patients with slightly more advanced disease (FIGO stages Ic, Ia<sub>ii</sub>, Ib<sub>ii</sub>, IIa, IIb).
- b. A series of ancillary papers is in preparation concerning further results from the early ovarian clinical trials. Efficacy and toxicity of P32 treatment has been analyzed and a paper has been submitted. Analyses restricted to stage II patients and to low malignant potential patients are both being completed and the results are being prepared for publication.

## 6. COLLABORATIVE RESEARCH WITH THE LUNG CANCER STUDY GROUP

- a. BRB staff has served as primary statistician for the following clinical trials:
  1. A protocol comparing CAP+RT vs. RT in patients with residual non-small cell lung cancer has been completed and demonstrated a modest survival advantage (and a greater time to recurrence advantage) for the CAP+RT treatment. Two papers have been published with Dr. Thomas Lad.
  2. A protocol comparing CAP vs. no treatment in patients with T<sub>1</sub>N<sub>1</sub> or T<sub>2</sub>N<sub>0</sub> NSCLC has been completed and analyzed and a paper is in preparation with Dr. Ronald Feld.
  3. A protocol comparing lobectomy vs. limited resection in T<sub>1</sub>N<sub>0</sub> NSCLC patients has completed accrual and is in follow-up under the supervision of Dr. Robert Ginsberg.
- b. BRB staff has acted as consultant statistician on a number of studies including development of protocols to evaluate the prognostic value of magnetic resonance imaging and supervision of an accrual survey. Analysis of the incidence of second primaries and recurrence among T<sub>1</sub>N<sub>0</sub> patients, across several protocols, has been completed and a paper is in preparation with Dr. Paul Thomas.

## 7. TIAZOFURIN TOXICITY

In collaboration with Dr. Jean Grem of the Investigational Drug Branch, clinical toxicity experience with Tiazofurin in the Phase I studies supported by CTEP was investigated. This review was motivated by a concern

regarding severe myelosuppression, but the results indicated that such toxicity was infrequent and not dose-dependent. A manuscript is in press.

8. EVALUATION OF SURAMIN FOR THE TREATMENT OF STAGE D2 CARCINOMA OF THE PROSTATE

BRB has collaborated with Dr. Michael Christian to organize extramural clinical trials to confirm the promising results reported by the NCI-COP for use of suramin in the treatment of patients with stage D2 carcinoma of the prostate who have failed hormone treatment. Patients with measurable disease will be enrolled in a phase 2 study. A randomized study will be performed for patients with non-measurable disease using survival, "quality-of-life" and biologic endpoints. BRB serves as statistical center for this study. The suramin dose will be determined by blood levels and BRB has collaborated with Dr. Charles Meyers on procedures for monitoring blood level assay calibration of the participating institutions.

9. META-ANALYSIS OF 5FU+MeCCNU IN THE TREATMENT OF CARCINOMA OF THE COLON

A meta-analysis of randomized trials evaluating 5fluoro-uracil and methyl CCNU for the adjuvant treatment of carcinoma of the colon is being conducted in collaboration with Drs. Michael Hamilton and Ho Chun.

10. PROGNOSTIC DETERMINANTS OF PATIENTS WITH NON-HODGKIN'S LYMPHOMAS

Follow-up information for the 1175 patients from 4 institutions used to develop the Working Formulation for Non-Hodgkin's Lymphomas was updated. A manuscript was published in the Annals of Internal Medicine on long-term survival results within the Working Formulation histologic subtypes. A second manuscript has been submitted on prognostic determinants for patients with diffuse large cell and immunoblastic lymphomas and a new staging system was proposed. A manuscript on prognostic determinants and staging of patients with low-grade lymphomas is in preparation. In conjunction with the usual regression methods, two new methods (recursive partitioning and cubic spline regression) have been used and appear to be promising tools for modelbuilding.

11. EVALUATION OF CRITERIA WHICH MAY PREDICT RESPONSE TO IL2/LAK TREATMENT

Preliminary data suggest that tumor cells that express Class II antigens (HLA-DR) may be more sensitive to LAK/IL2. Tumor specimens analyzed in the surgical pathology department of five institutions participating in LAK/IL2 trials are being evaluated for expression of HLA-DR. The reliability of the technique (agreement within and between observers) is being studied. The project will also provide an estimate of the frequency of HLA-DR for different histologies, and will determine whether expression of HLA-DR can be predicted by other morphologic features. Relation of HLA-DR expression to response to LAK/IL2 treatment is the final goal of this study.

## 12. DATA MONITORING COMMITTEES

The BRB has collaborated with the Clinical Investigations Branch (CIB) to ensure that all CTEP supported cooperative group studies that involve control arms with no treatment after surgery are carefully monitored by data monitoring committees. CTEP has supported the use of data monitoring committees for all phase 3 multi-center studies and has required them for the type of studies specified above. BRB and CIB developed guidelines for the operation of these committees and serve on them.

## 13. INTERGROUP STUDIES

- a. With the input of CTEP staff and the extramural community of cancer cooperative group investigators, the BRB has developed a set of guidelines for the conduct of studies involving two or more cooperative groups. For scientific as well as financial reasons, the number of intergroup studies has rapidly increased. In the past, intergroup studies have generally been developed and conducted in an informal manner. Many participants in intergroup studies have been frustrated by the lack of adequate quality control mechanisms, opportunities for input to study design, and regular monitoring reports. At the request of the cooperative group chairmen, and with input from the Clinical Investigations Branch, we have developed a set of guidelines for the design and conduct of intergroup studies.
- b. Facilitating the conduct of intergroup studies is an important priority of CTEP. The intergroup guidelines should contribute to this. The BRB is also working with group statisticians to determine, for several test intergroup studies, whether the amount of data collected can be substantially reduced without interfering with the quality or value of the research. Reduction in amount of data collection would both reduce costs and reduce the complexity of intergroup studies.
- c. BRB staff has worked with the coordinating centers of the two pediatric cooperative groups to develop effective procedures for pediatric intergroup studies. Such procedures have now been agreed upon and implemented.
- d. BRB worked with cooperative group data managers to develop a national workshop on intergroup studies which was held in Denver, Colorado on June 20-21, 1987. More than 150 representatives of the cooperative groups participated in this workshop. Discussion topics included general issues in intergroup studies as well as specific problems in ongoing studies. The proceedings were prepared and circulated to the cooperative groups by BRB staff with the assistance of CTEP contractors. BRB is representing CTEP in the planning of a second intergroup study workshop in the spring of 1990. A planning meeting of cooperative group data coordinators will be held in Bethesda in September 1989.

- e. BRB worked with cooperative group statisticians in the development of guidelines for steering committees for intergroup studies

#### 14. ADVERSE DRUG REACTIONS

In collaboration with Dr. Leyland-Jones we have evaluated the incidence of adverse drug reactions resulting from special exception drug access compared to use of the same drugs in research protocols.

#### 15. DRUG DEVELOPMENT REVIEWS

The clinical trials conducted in the development of CHIP and CBDCA have been reviewed and an assessment of lessons learned for the development of future analogs has been performed in conjunction with Dr. Brenda Foster. A manuscript is in press. A manuscript with Dr. Foster has been published based on a review of in-vitro data on the modulation of adriamycin resistance. A review of the clinical trials conducted in the development of AMSA has been performed with Dr. Leyland-Jones. A manuscript has been submitted on this case-study in the development of an anti-leukemia agent. A review of clinical trials with deoxycoformycin for patients with hairy cell leukemia who have failed interferon has been performed with Dr. Cheson. A manuscript has been accepted for publication.

#### 16. GROUP C/TREATMENT IND PROTOCOLS

In order to make effective drugs available to the oncologic community as early as possible, the CTEP has utilized the group C and Treatment IND categories of the Food and Drug Administration. In order to obtain information on the effectiveness and toxicity of these drugs when used outside of research protocols, data are collected for these patients. The extent of data collection varies substantially by drug. The BRB has statistical responsibility for these protocols. Three protocols have been developed to date:

R88-0001: Treatment of patients with refractory germ cell carcinoma with Cisplatin, Etoposide (or Vinblastine), Ifosfamide and Mesna.

R88-0002: Pentostatin in patients with active hairy cell leukemia previously treated with alpha-Interferon.

R88-0003: VM-26 in combination with ARA-C for the treatment of patients with relapsed acute lymphoblastic leukemia.

FAMP for patients with refractory chronic lymphocytic leukemia.

Methyl CCNU for patients with resectable adenocarcinoma of the colon or rectum.

Levamisole for use with 5-FU as adjuvant treatment for patients with Dukes C adenocarcinoma of the colon.



17. EVALUATION OF DFMO AND MGBG FOR THE TREATMENT OF ANAPLASTIC ASTROCYTOMAS

BRB collaborated with Drs. Michael Christian, and Victor Levin and with the Merrill Dow Corporation to develop potential licensing studies for these polyamine inhibitors.

18. PLANNING OF MULTI-TREATMENT CLINICAL TRIALS

Clinical trials with more than two treatment arms often require a more complex analysis strategy than do two-arm trials. For example, a recent CTEP sponsored clinical trial NSABP B21, involves randomization of patients with occult breast cancer primaries to receive either breast irradiation, tamoxifen or both. The treatment of choice will be tamoxifen alone if it is better than XRT alone and no worse than the combination. Similarly for breast irradiation alone. The combination is the treatment of choice if it is better than both single modality regimens. Traditional methods for planning clinical trials do not take into account such compound decision criteria. We have performed sample size calculations that account for decision strategies for such clinical trials. These results have been used for the planning of other studies such as the NCCCTG 88-24-53 four arm evaluation of thoracic irradiation and chemotherapy for patients with stage 2-3a non-small-cell-lung cancer.

19. BAYESIAN MODEL FOR EVALUATING WHETHER TREATMENT DIFFERENCES VARY AMONG SUBSETS

One of the most difficult and important aspects of interpreting major comparative clinical trials is the evaluation of whether relative treatment efficacy varies substantially among subsets of patients defined with regard to baseline characteristics. Conventional statistical procedures for evaluating such "treatment by subset interactions" are notoriously conservative when the number of subsets is large. We have developed two new statistical approaches to this problem. In one approach we use the Bayesian notion of a-priori exchangeability of interactions and a non-informative prior for the unknown variance component. Consequently, the result of the analysis is not subjective and does not require the elicitation of prior beliefs. The methods is easily applied to the results of proportional hazards or logistic models and we have developed a computationally efficient algorithm for calculating posterior distributions of interaction terms and subset specific treatment effects utilizing decomposition methods. We have re-analyzed the rectal cancer adjuvant clinical trial (R01) of the National Surgical Adjuvant Breast and Bowel Cancer Project using this method. A manuscript has been submitted for publication. In the other approach we permit the specification of a subjective prior for the magnitude of a specific treatment by subset interaction. This approach was illustrated in a NEJM letter describing our re-analysis of a multi-center trial of diltiazem for myocardial infarctions.

## 20. SUBSET ANALYSIS

BRB staff have investigated the problem of misleading results arising from analysis of patient subsets in clinical trials, have reviewed statistical tools for more reliable subset analyses and have prepared a set of recommendations to clinical trials investigators concerning the conduct and reporting of such analyses. A manuscript is in preparation.

## 21. STATISTICAL PLANNING OF "PHASE II" STUDIES OF COMBINATION REGIMENS

Phase II clinical trials of new drugs determine whether there is any anti-tumor activity in the disease studied. The objectives of phase II studies of combinations of active drugs are more ambitious. Such studies generally seek to determine whether the level of activity is sufficient to warrant a randomized phase III trial. Such phase II trials are inherently comparative, with historical experience on standard treatments being the basis for informal comparison. The prevalence of negative phase III trials is indicative of the inadequacy of many phase II trials. We have attempted to improve the planning of phase II trials of active combinations by explicitly incorporating specific historical control data. Planning accounts for the finite size of the historical series' and for the degree of inter-study variability among historical control results. We find that with a substantial historical control experience and little inter-study variability, the conventional phase II trial is appropriate. In other circumstances, however, the conventional approach is found to provide a high probability of false positive and false negative results; larger sample sizes and a proportion of the patients randomized to the standard treatment for combining with historical controls are required. A manuscript describing these results has been accepted for publication.

## 22. DESIGN OF DOSE ESCALATION SCHEMES IN PHASE I STUDIES

Simulations have been conducted as part of an ongoing project to develop more efficient dose escalation schemes for phase I studies (to define the maximal tolerated dose) and to characterize the statistical properties of these designs. We have been particularly concerned about the adequacy of traditional phase I designs for dose escalation of combinations in the presence of bone marrow growth factors. The maximum tolerated doses defined in such studies may be used directly in phase III trials.

## 23. SAMPLE SIZE CONSIDERATIONS FOR STUDIES COMPARING SURVIVAL CURVES USING HISTORICAL CONTROLS

It is generally impractical to test all new regimens in randomized comparative trials; preliminary screening based on non-randomized pilot investigations is necessary. In addition, for some rare diseases historically controlled comparisons are the only possibility. Although there is an extensive literature on specification of sample size for

randomized clinical trials, this is not the case for studies involving historical controls. The required sample size depends on the extent and follow-up maturity of the historical data base. We have developed methods of sample size planning for historically controlled studies when the primary endpoint is survival or disease-free-survival. A manuscript describing these results has been published.

#### 24. CUBIC SPLINE REGRESSION

Continuous variables are problematic in the development of predictive models. Most often a simple linear or quadratic effect is assumed. In some cases, however, there may be a more complex relationship between response and the continuous covariate and it is desirable not to impose a standard form but rather to discover the nature of the relationship. We have investigated the use of restricted cubic splines in such models, described how to use standard statistical software for fitting such models and compared this approach to the standard approaches and to other nonparametric methods. A manuscript has been published describing these results. We are performing a multivariate analysis of prognostic factors for primary breast cancer using this methodology in collaboration with Dr. Carol Redmond of the National Surgical Adjuvant Breast and Bowel Project.

#### 25. DESIGN OF PHASE II CLINICAL TRIALS

We have reviewed the statistical approaches to the design of single agent phase II trials, and have reviewed the response rates found in such trials for drugs tested since 1975. Recommendations concerning the size, design and number of such trials for a drug have been developed. A new two-stage design for phase II trials has been developed which is optimal in the sense that the expected number of patients accrued is minimized. Two manuscripts describing this research have been published.

#### 26. SELECTION OF THE MOST EFFECTIVE TREATMENT

The BRB has conducted research on two-stage clinical trials designs. During the first stage several experimental treatments are examined. The most promising treatment is selected and then during the second stage that experimental treatment is compared to a standard therapy. Provision for termination after the first stage if no experimental treatment is sufficiently promising and for possibly including the standard treatment in the first stage have also been evaluated. The operating characteristics of these designs have been studied and their parameters optimized for most efficient utilization of patients. These provide a more reliable basis for selecting the experimental regimen to be evaluated in a large phase III trial than is available from uncontrolled pilot studies. These designs are appropriate where there are several experimental treatments of interest but insufficient patients to evaluate them all in a phase III trial. Three manuscripts describing this work have been either published or are in press.

## 27. QUANTIFYING PREDICTIVENESS OF MULTIVARIATE SURVIVAL MODELS CONTAINING COVARIATES

Identification of factors that predict the prognosis of cancer patients is important for advising patients, improving the efficiency of clinical trials and for more effectively targeting important therapeutic questions to appropriate subsets of patients. Many prognostic models are "statistically significant" but not very predictive. We have developed measures to quantify the importance of such models for predicting survival or disease-free survival. Such quantification is a useful step in the development of truly accurate predictors. A manuscript describing this work has been accepted for publication.

## 28. SEQUENTIAL ANALYSIS OF CLINICAL TRIALS

The BRB has been conducting research on several aspects of the use of sequential analysis to enhance the efficiency of clinical trials. As treatments for various diseases improves, studies are undertaken in order to develop new therapeutic approaches that would be as efficacious, and less toxic. When the new treatment is more conservative than the standard, proper design of the study should insure that adoption of the new treatment will not result in an unacceptable loss in efficacy. Such trials require large numbers of patients. We have developed a group-sequential approach for planning and monitoring these trials and show that the reduction in sample size can be substantial. A manuscript has been submitted for publication. This approach has been presented to a national statistical meeting and as an invited seminar at the Food and Drug Administration.

## 29. RELATIONSHIP OF RECURRENCE TO SURVIVAL IN LARGE BOWEL CANCER

Survival is the primary endpoint of many major adjuvant clinical trials of large bowel cancer. For planning follow-on trials, however, time till first recurrence is often used as a surrogate endpoint. We are retrospectively analyzing data from major clinical trials that showed a treatment effect on time to recurrence in order to evaluate the relationship between recurrence time and survival.

## 30. MODEL SELECTION IN STEPWISE REGRESSION

Stepwise regression is one of the most commonly used methods of data analysis in statistics, including biostatistics. The commonly used methods for deciding when to terminate the stepwise procedure are ad-hoc, however. In collaboration with Drs. Peter Thall and David Greer of George Washington University, we are evaluating the use of cross validation and generalized cross validation as model selection criteria in stepwise regression.

### 31. WHEN TO RANDOMIZE?

Some clinical trials compare two therapeutic approaches which differ only after a certain time. A typical example is chemotherapy alone compared to chemotherapy followed by radiotherapy. Controversy exists about the optimal time to randomize, whether it should be before starting chemotherapy, or after its completion. Late randomization avoids the loss of power due to drop-outs after randomization. But early randomization is often more comfortable to physicians and results in fewer patients refusing participation. We have developed a model comparing adequate sample sizes with each design in order to provide guidelines in these situations. A manuscript has been submitted for publication.

### 32. CTEP INFORMATION SYSTEMS PLANNING

Dr. Simon serves as chairman of the CTEP computer committee. During this year major new system plans have been adopted for information management in the Protocol and Information Office and for the Drug Management and Authorization Section of the Investigational Drug Branch.

#### STAFF PUBLICATIONS:

Dixon DO, Simon R. Sample size considerations for studies comparing survival curves using historical controls. *J Clin Epid* 1988;41:1209-1214.

Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med* 1989;8:551-562.

Durrleman S, Simon R. The effect of diltiazem on mortality and reinfarction after myocardial infarction (letter). *New Eng J Med* 1989;320:123.

Foster BJ, Harding BJ, Wolpert-DeFilippes MK, Rubinstein LV, Claggett-Carr K, Leyland-Jones B. A strategy for the development of two clinically active cisplatin analogs: CBDCa and CHIP. *Cancer Chem Pharm* (in press).

Korn EL, Simon R. Measures of explained variation for survival data. *Stat Med* (in press).

Mathers FJ, Simon R, Clark GM, VonHoff DD. A method for the evaluation of dose-toxicity relationships in clinical trials. *Stat Med* (in press).

Paull KD, Hodes L, Shoemaker RH, Monks A, Scudiero DA, Rubinstein L, Alley MC, Plowman J, Boyd MR. The display and analysis of patterns of differential growth inhibition by drugs against a panel of human tumor cell lines. *J Nat Can Inst* (in press).

Sadeghi A, Payne D, Rubinstein L, Lad T. Combined modality treatment for resected advanced non-small cell lung cancer: local control and local recurrence. *Int J Rad Oncol Biol Phys* 1988;15:89-98.

Simon R. A critical assessment of approaches to improving the efficiency of cancer clinical trials. In *Recent Results in Cancer Trials* (Baum M, Kay R, Scheurle H. Eds.). Springer-Verlag 1988.

Simon R. Efficient designs for clinical trials. *Oncology* (in press).

Simon R. Interim analysis: the repeated confidence interval approach (discussion) *J Royal Stat Soc B*. (in press).

Simon R. Optimal two-stage designs for phase II clinical trials. *Controlled Clin Trials* 1989;10:1-10.

Simon R. Publication Bias: A problem in interpreting medical data (discussion). *J. Royal Stat Soc B* (in press).

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 CM 06308-18 BRB
<b>PERIOD COVERED</b> October 1, 1988 through September 30, 1989		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders )</b> Biometric Research Branch		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)</b> Richard M. Simon, Ph.D., Chief, Biometric Research Branch, CTEP, DCT, NCI Others: Lawrence V. Rubinstein, Ph.D., Statistician, BRB, CTEP, DCT, NCI Sylvain Durrleman, M.D., Statistician, BRB, CTEP, DCT, NCI Timothy Chen, Ph.D., Statistician, BRB, CTEP, DCT, NCI Edward Korn, Ph.D., Statistician, BRB, CTEP, DCT, NCI		
<b>COOPERATING UNITS (if any)</b> Developmental Therapeutics Program, DCT, NCI; Radiation Research Program, DCT, NCI; Biological Response Modifiers Program, DCT, NCI; Clinical Oncology Program, DCT, NCI; Environmental Epidemiology Branch, DCE, NCI; George Washington University (Peter Thall, Ph.D.); M.D. Anderson Tumor Institute (Dennis Dixon, Ph.D.)		
<b>LAB/BRANCH</b> Biometric Research Branch		
<b>SECTION</b>		
<b>INSTITUTE AND LOCATION</b> NCI, NIH, Bethesda, Maryland 20892		
<b>TOTAL MAN-YEARS</b> 4.75	<b>PROFESSIONAL</b> 3.5	<b>OTHER</b> 1.25
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided )</b>  <p>The Biometric Research Branch (BRB) is the statistical component for scientific planning and monitoring of the national and international research program of the Division of Cancer Treatment. The branch provides statistical leadership for all extramural activities of the division. The branch is also responsible for statistical consultation and collaboration with the intramural activities of the Biological Response Modifier Program and the Developmental Therapeutics Program.</p> <p>The Biometric Research Branch performs statistical planning and evaluation of all Division of Cancer Treatment supported therapeutic clinical trials. The branch performs scientific monitoring and analysis of extramural trials. Primary statistical direction is provided by the branch for the conduct of selected national and international studies of therapeutic interventions, prognostic factors, pre-clinical screening and diagnostic imaging. The branch performs evaluations of therapeutic interventions based upon syntheses of results from multiple studies.</p> <p>The Biometric Research Branch conducts research on experimental designs, biometric methods and biomathematical approaches for the development and efficient evaluation of improved cancer treatments.</p>		





## CLINICAL INVESTIGATIONS BRANCH

### GENERAL BACKGROUND

The Clinical Investigations Branch (CIB) is responsible for the administration and coordination of the extramural clinical trials sponsored by the Division of Cancer Treatment. In performing this task, the CIB coordinates its activities with the other Cancer Therapy Evaluation Program Branches and with relevant NCI and NIH components. Utilizing an integrated mixture of advisory, informational, and facilitative activities, CIB identifies promising scientific opportunities, and follows up by stimulating specific multi-institutional trials. The CIB emphasizes rigorous prioritization of research questions leading to clinical trials since the number of worth questions is greater than can be addressed by the existing clinical trials network. The need to concentrate on key issues and to address them with trials of adequate size done in an expeditious manner is paramount and governs the activities of the CIB.

### COMPREHENSIVE DISEASE/MODALITY INFORMATION

In order to promote efficient collaborative research, the CIB utilizes a comprehensive disease and modality perspective to identify and articulate key research questions, the CIB staff actively gather disease and modality information from all available sources, including the published literature, interim data from domestic and foreign cooperative groups, information from scientific meetings, and the pool of research project grants supported by the NCI.

Each individual staff member of CIB is responsible for maintaining information on current and developing research opportunities and serves as an information resource to CTEP, DCT and extramural investigators, as follows:

#### CIB MODALITY COORDINATORS

<u>MODALITY</u>	<u>STAFF</u>
BONE MARROW TRANSPLANT	CHESON
INFECTIOUS DISEASE	CHESON
NUTRITION	MOORE
PSYCHOSOCIAL	MOORE
RADIATION	HAMILTON
SURGERY	FRIEDMAN (interim)

Specific disease responsibilities are divided as follows:

<u>DISEASE</u>	<u>STAFF</u>
AIDS	CHESON
BRAIN	HAMILTON
BREAST	DORR
ENDOCRINE	HAMILTON
GASTROINTESTINAL	HAMILTON
GENITOURINARY	DORR
GYNECOLOGIC	MOORE

## Disease Responsibilities--Continued

<u>DISEASE</u>	<u>STAFF</u>
HEAD & NECK	MOORE
LEUKEMIA (ADULT)	CHESON
LUNG	MOORE
LYMPHOMA	CHESON
MELANOMA	DORR
MYELOMA	CHESON
PEDIATRIC (LEUKEMIA + SOLID)	UNGERLEIDER
SARCOMA	HAMILTON

## COORDINATION AND ADMINISTRATION OF THE COOPERATIVE GROUP SYSTEM

A major responsibility of the Clinical Investigations Branch is to advise and coordinate administrative and scientific aspects of the Clinical Cooperative Groups. This effort is necessary to optimize the productivity of the cooperative agreement mechanism (U10), through which the NCI provides funds for definitive (Phase III) multi-institutional trials. Approximately \$60 million is devoted to this mechanism. The CIB is responsible for and responsive to the Cooperative Groups; peer review judges the ultimate product. While the CIB has interest in administrative and scientific aspects of the Groups, it is not concerned with their micromanagement.

The Clinical Investigations Branch advises and directs the Cooperative Groups in allocating limited financial, physician and patient resources. During the past year, particular group-related administrative activities have included: supervision of the implementation of a per-case reimbursement system by the Brain Tumor Cooperative Group during the period following NCAB approval; provision of interim funding for the Gynecologic Oncology Cooperative Group, and supervision of that Group's reorganization utilizing a patient capitation financial structure and subsequent submission of an amended competing application; redefining the terms of award for cooperative agreements (accepted by the Group Chairmen and by the DCT Board of Scientific Counselors and currently under NIH review); advising the Radiation Therapy Oncology Group concerning its competing reapplication, integrating per-case reimbursement in its financial organization; supervision of the phase-out of the Leukemia Intergroup study providing administrative supplementation to the Eastern Cooperative Oncology Group for continued supervision of the Intergroup Melanoma study; and supervision of the selection of the next generation of high priority clinical trials by the Cooperative Group Chairmen. These activities were in addition to the more routine administrative activities of devising and implementing a funding plan for successfully recompeting Groups and institutions, using available funds, which represented a fraction of the amount recommended by peer review.

From a scientific point of view, the CIB and the Cooperative Group system identify and prioritize clinical research questions of interest. There is a potential interaction between the CIB and all Group organizational levels at any time during the process of generating a study, as follows:

CIB staff regularly attend formal Group meetings to serve as a source of information and to provide guidance in the development of protocols. An effort is made to prevent duplicative protocols and to foster the very best science.

CIB staff organize strategy meetings in selected disease sites in order to help provide an overview of current therapeutic issues whose resolution might be facilitated through collaborative clinical trials. Representatives of cooperative groups participate in these meetings in which a consensus regarding the objectives and design of optimal trials is developed. The likelihood of duplicative trials is reduced and the probability of intergroup trials is enhanced by this process.

An increasingly important area of interaction is the Concept Review, an evaluation of the essence of a major Phase III study proposal while still in an early stage of development as it is deemed more efficient and productive to evaluate a concept than to modify a protocol at the final stage of development. A description of the content of a concept submission was developed by CIB staff to ensure uniformity. A brief document outlining the scientific background, objectives, eligibility, treatment schema and statistical section is sent by the investigators to the CIB, which provides relevant criticism in return. During the past year, 19 concepts were reviewed, of which 5 went forward to become active studies; one is currently in review. This format invites fruitful early dialogue between the investigators and NCI, at a time when the thrust of the experiment is most easily altered.

The formal Protocol Review process is in itself a major analytic activity. In this forum, a mature study plan that has already undergone considerable Group discussion and assessment is reviewed for safety and scientific issues. CTEP staff critique these protocols and request changes when appropriate. In order to supplement intramural expertise outside reviewers assist as needed. A written consensus review is provided the investigators which outlines required and/or recommended changes in the protocol document.

A continuum of CIB interaction with the Cooperative Group system from the very earliest idea formation to the review of the finished document consequently exists throughout the evolution of a protocol.

The CIB promotes clinical trials that are sufficiently large to be reliable, and are completed in the briefest possible time. The CIB encourages appropriate intergroup studies. Generally, at any particular moment, there are a limited number of scientific questions of the highest priority. An intergroup study is deemed appropriate when a study by an individual Cooperative Group would require an inordinately long time for completion and/or might accrue too few patients to permit statistically valid conclusions.

Finally, the CIB promotes relevant laboratory-clinical correlative investigations which might prove scientifically fruitful. Information concerning the best correlative studies comes not only from Group pilot activities, but also from information gained from the R01/P01 pool of grants which CIB manages.

The following is a list of the Cooperative Group organizations that were functioning with NCI support in FY89 and the CIB staff member who was responsible for scientific liaison with that organization.

<u>GROUP</u>	<u>CIB STAFF</u>
Brain Tumor Study Group (BTSG)	Hamilton
Cancer and Acute Leukemia Group B (CALGB)	Cheson
Children's Cancer Study Group (CCSG)	Ungerleider
Eastern Cooperative Oncology Group (ECOG)	Dorr
European Organization for Research on Treatment for Cancer (EORTC)	Cheson
Gynecologic Oncology Group (GOG)	Nerenstone-->Moore
Intergroup Melanoma Group (IMG)	Nerenstone-->Dorr
Intergroup Rhabdomyosarcoma Study (IRS)	Ungerleider
Intergroup Sarcoma Group (ISG)	Nerenstone--
>Hamilton	
Lung Cancer Study Group (LCSG)	Moore
National Surgical Adjuvant Breast and Bowel Project (NSABP)	Dorr
National Wilms' Tumor Study Group (NWTG)	Ungerleider
North Central Cancer Treatment Group (NCCTG)	Hamilton
Pediatric Oncology Group (POG)	Ungerleider
Quality Assurance Review Center (QARC)	Hamilton
Radiation Therapy Oncology Group (RTOG)	Hamilton
Southwest Oncology Group (SWOG)	Cheson

#### COOPERATIVE GROUP OUTREACH PROGRAM (CGOP)

The Cooperative Group Outreach Program was transferred from DCPC to DCT in FY87, at which time a recompetition for awards was conducted. Five Groups (Eastern Cooperative Oncology Group, Children's Cancer Study Group, Cancer and Leukemia Group B, Southwest Oncology Group and the National Surgical Adjuvant Breast and Bowel Project) were selected for awards in FY88. As planned at the time of recompetition, the CGOP award periods are synchronous with the parent cooperative group award and require recompetition at the time the parent group recompetes. In FY89, the Children's Cancer Study Group successfully recompleted and had its CGOP component approved for an additional 5 years.

#### SCOPE OF GROUP ACTIVITIES

In 1985 approximately 18,000 new patients were entered on therapeutic studies (Phase I, II and III) in CTEP sponsored trials conducted by the Cooperative Groups. By 1988, accrual of group treatment studies had increased to over 22,000 with most of these patients entering Phase III trials (Figure A). Virtually every type of malignancy is being studied in this collaborative enterprise. Phase II/III estimates of activity and definitive tests of efficacy are the central components of the effort to reduce cancer mortality. Patient accession by disease is indicated by Figure B.

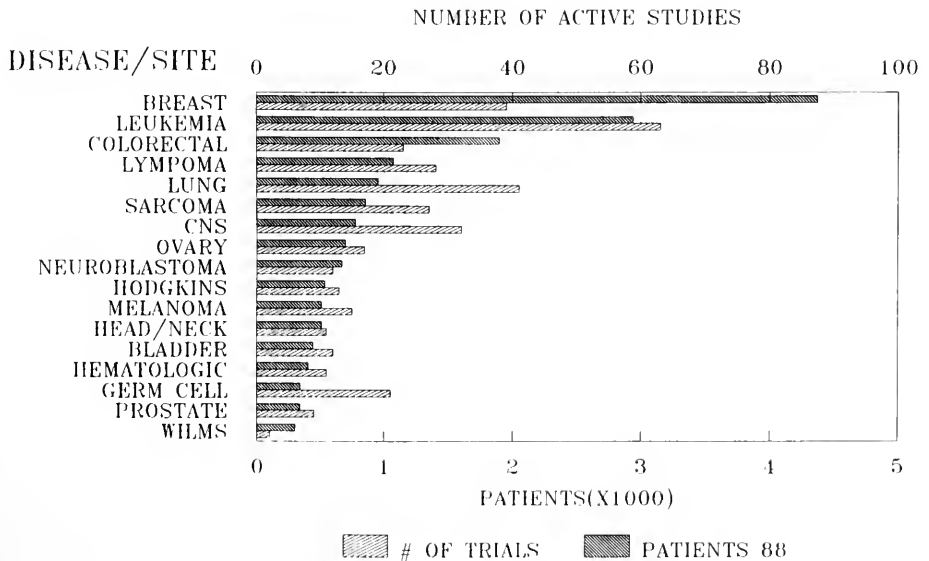
FIGURE A

NCI CLINICAL COOPERATIVE GROUPS  
ACCUAL SUMMARY  
 CALENDAR YEAR 1988

	PATIENT ENTRIES	OPEN STUDIES
PHASE I	602	34
PHASE II	5,463	310
PHASE III	15,057	183
NON-THERAPEUTIC	11,115	67
EORTC (1987)	5,678	202

FIGURE B

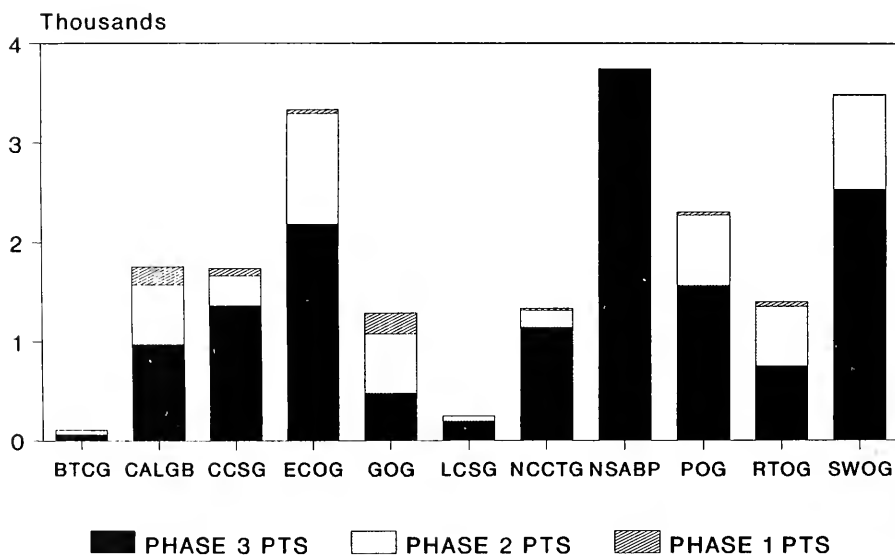
PHASE II & III GROUP TRIALS  
 ACTIVITY BY DISEASE/SITE - 1988



Accrual to treatment studies by the major Cooperative Groups is shown in Figure C:

FIGURE C

## NCI CLINICAL COOPERATIVE GROUPS ACCRUAL SUMMARY - 1988



In relation to a number of specific causes of cancer deaths, the following table compares impact of disease and clinical research effort.

COOPERATIVE CLINICAL GROUP STUDIES IN SELECTED  
DISEASE AREAS - PROTOCOLS ACTIVE IN 1988  
ACCRUAL TO PHASE II AND PHASE III STUDIES

ORGAN SYSTEM *****	NEW CASES IN 1988 *****	ALIVE AFTER FIVE YEARS *****	STUDIES OPEN TO ACCRUAL *****	TOTAL ACCRUAL 1988 *****	% ONTO GROUP STUDIES *****	STUDIES IN ADVANCED DISEASE *****	PATIENTS W/ADVANCED DISEASE *****	ADJUVANT STUDIES *****	PATIENTS W/CURATIVE POTENTIAL* *****
BREAST	131619	86899	39	4374	3.3	24	808	15	3566
CERVIX	14425	8784	23	405	2.8	18	228	5	177
COLON/RECTUM	141053	58023	22	1891	1.3	12	636	10	1255
ESOPHAGUS	9616	400	6	73	0.8	4	28	2	45
LUNG	137837	14817	43	1492	1.1	41	1440	2	52
MYELOMA	11000	2993	11	405	3.7	11	405	0	0
PANCREAS	25229	535	9	200	0.8	9	200	0	0
PROSTATE	90532	44358	11	420	0.5	7	252	4	168
STOMACH	14897	3131	10	159	1.1	9	142	1	17
*** Total ***	576208	219940	174	9419		135	4139	39	5280

\* PATIENTS RECEIVING ADJUVANT THERAPIES

## TERMS OF AWARD FOR GROUPS

The formal rules describing the interaction of the NCI and the Cooperative Groups and the expectations for Group performance are known as the Terms of Award. These Terms have been revised by CIB staff to reflect evolving expectations. Under the new Terms, the NCI will interact more closely with each Group to implement clinical studies of the highest quality. New studies will be initiated only after much closer scrutiny to insure that all relevant scientific issues have been considered and that the study will be completed as quickly as possible. Intergroup collaborations will be required where appropriate, and capitation forms of reimbursement utilized to stimulate patient enrollment. The substantial NCI involvement called for by the cooperative agreement mechanism will be enhanced in order to maximize productivity. The new Terms of Award are currently undergoing NIH review, having been accepted by the Cooperative Group Chairmen and endorsed by the DCT Board of Scientific Counselors.

## HIGH PRIORITY CLINICAL TRIALS--THE NEED TO INCREASE ACCRUAL

A major impediment to progress in curing more cancer patients is that the necessary clinical investigation proceeds too slowly. For nearly every malignant disease, crucial studies accrue patients at an unsatisfactory rate, thus preventing the identification of new effective therapies in a timely and precise fashion. The Clinical Cooperative Groups are major contributors to clinical research, and currently enroll about 22,000 patients/year on

therapeutic studies. However, this number is tiny when compared to the roughly 1,000,000 new cancer cases identified yearly in the USA. For the common adult malignancies only .5% to 3% of available patients are studied each year. Since definitive studies may require 1000 to 3000 patients, it has often taken a decade or more to complete the accrual phase of a study. With the dramatic expansion of basic and applied scientific research, there are an unprecedented number of research options.

Faced with this situation, in FY88 CTEP organized a new initiative to increase accrual to cooperative group trials. Five protocols for four potentially curable diseases--adjuvant colon, rectum, bladder and advanced lymphoma were been designated as "High Priority Clinical Trials" of national importance and are targeted for special attention.

These studies are likely to provide important new information and to have an impact on national mortality rates. In order to succeed, there must be greater awareness of and enthusiasm for clinical trials by the general public and health care deliverers.

Efforts to increase accrual to designated "High Priority Clinical Trials" are progressing along two parallel tracks:

- a. The Office of Cancer Communications (OCC) is coordinating assessment and information campaigns for the lay and professional communities. The general public is being educated about clinical trials via print and electronic media. The various Cancer Information Services are also being targeted for OCC attention. The aim of this effort is to stimulate lay enthusiasm for volunteering for protocol studies.
- b. The multidisease, adult Cooperative Groups are expanding their clinical bases. More than 4000 American Society of Clinical Oncology (ASCO) member physicians were contacted and hundreds responded to the invitation to participate in the High Priority Trials. After screening, about 157 practices or institutions (new and/or currently unfunded) were identified as promising resources. Four Groups submitted detailed proposals to enhance accrual to the selected trials and received supplementation of their awards in FY88 and 89 to provide financial reimbursement for the costs of accruing additional patients.

The impact of this enhanced accrual to adjuvant studies of patients with colon, rectum, and bladder cancer and for those with aggressive stage III-IV lymphomas will likely be substantial. A brief description of these trials is as follows:



# HIGH PRIORITY TRIALS

STUDY	DISEASE	STUDY DESIGN
Intergroup 0067	High-Grade Lymphoma	CHOP vs. M-BACOP, ProMACE-Cytacom vs. MACOP-B
Intergroup 0080	Bladder (Adjuvant)	Cystectomy vs. M-VAC + Cystectomy
NCCTG 86-47-51	Rectal (Adjuvant)	5-FU (CI or bolus) + RT vs. 5-FU (CI or bolus) + methyl-CCNU + RT
NSABP C-03	Colon (Adjuvant)	5-FU/Leucovorin vs. MOF
NSABP R-02	Rectal (Adjuvant)	MOF +/- RT vs. 5-FU/Leucovorin +/- RT

As of June 1989, four of the five Phase III trials are accruing patients (the colon adjuvant trial progressed at three times its projected rate and closed in April 1989. The lymphoma and NSABP rectal studies are accruing at well above the projected rate. The NCCTG rectal trial and the intergroup bladder trial are entering patients at the originally projected rates. The annualized accrual rate for January 1-April 1, 1989 has increased relative to the previous quarter for each high priority trial and overall is 20% higher (1528 vs. 1292).

# HIGH PRIORITY TRIALS

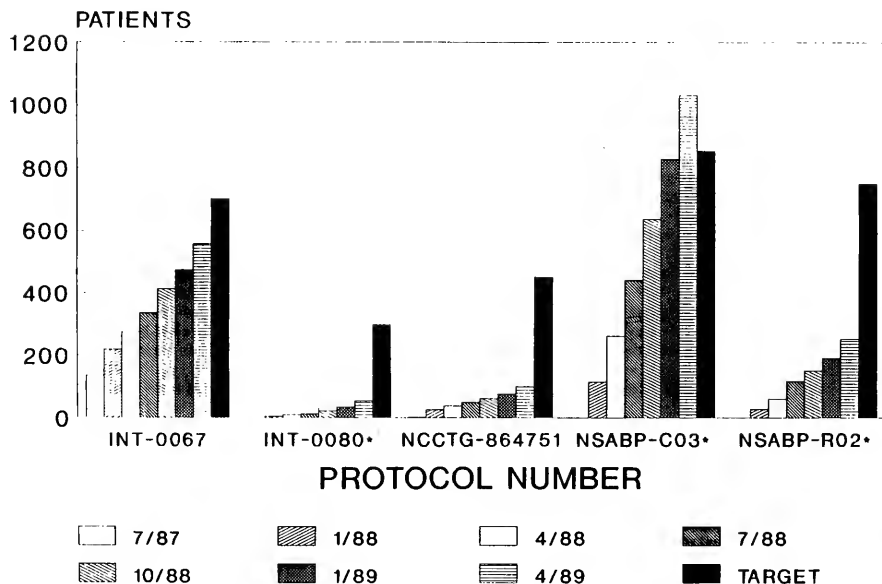
## ACCRUAL HISTORY

STUDY	ACCRUAL	7/1/87	1/1/88	7/1/88	1/1/89	4/1/89
Lymphoma (INT 0067)	700*	136	219	336	473	556
Bladder (INT 0080)	298	0**	5	12	35	54
Rectal (NCCTG 86-47-51)	450	1	26	49	76	100
Colon (NSABP C-03)	855	0**	114	441	830	1033
Rectal (NSABP R-02)	750	0**	27	117	191	252

\*Revised from 824.

\*\*Study activated after 7/1/87.

# ACCRUAL TO HIGH PRIORITY TRIALS



• STUDY ACTIVATED AFTER 7/87

An additional six studies have been nominated for high priority trial status for FY90 by the Cooperative Group Chairmen and are currently under review by the DCT Board of Scientific Counselors, whose endorsement is required prior to designation. These studies may be summarized as follows:

STUDY	DISEASE	CURATIVE INTENT	LABORATORY CORRELATIONS	CLINICAL HYPOTHESIS
INT 0089	Colon (Dukes' B/C)	Yes Adjuvant	Yes Laminin Binding Molecular Genetics Drug Resistance	Biochemical Modulation of 5-FU by Folinic Acid
RTOG 8808	NSC Lung (Stage III)	Yes	No	"Neoadjuvant" Chemotherapy and Chemotherapy-Radiation Interactions
NSABP B-18	Breast (Stage I-II)	Yes Adjuvant	Yes Ploidy, ER-ICA	"Neoadjuvant" Chemotherapy
NSABP B-21	Breast (Occult)	Yes Adjuvant	No	Extent of Therapy Necessary
INT-0096	SC Lung (Limited)	Yes	No	Accelerated Dose Radiation
INT-0102	Breast (Node Neg)	Yes Adjuvant	Yes Ploidy, Oncogene, S Phase	Chemotherapy Comparisons and Natural History Data

#### CLINICAL TRIALS TRACKING SYSTEM

CIB has developed a clinical trials tracking system to follow the progress of ongoing studies and to coordinate subsequent trials. This system draws on designated items of high interest in the current CTEP information system, made more accessible through a data base management system. Major features of this system are the ability to organize and identify studies by the scientific hypothesis being tested, to accurately assess patient accrual and projected closure date, thus improving the management of Group studies.

## STRATEGY MEETINGS

Strategy meetings help provide an overview and prioritize national efforts in selected disease sites. Expert oncologists from the Cooperative Group and Cancer Centers meet at the National Cancer Institute to review ongoing clinical experiments and identify short-term priorities for research. The format of these meetings is to review the ongoing Cooperative Group clinical trials (with current estimates of accrual and projections of when studies would be completed) with discussion devoted to strategies for the next generation of clinical trials. Where appropriate, intergroup efforts are encouraged in order to achieve greater economy and statistical power. These meetings result in considerable exchange of information. The following topics have been discussed.

### CLINICAL INVESTIGATIONS BRANCH FISCAL YEAR 1988 STRATEGY MEETINGS

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**TOPIC:** ESOPHAGEAL CARCINOMA

**DATE:** January 23, 1989 [Squamous Carcinoma]  
January 24, 1989 [Adenocarcinoma]

**COORDINATOR:** J. Michael Hamilton, M.D.

<u>Participants</u>	<u>Cooperative Group/Institution</u>
Joel Tepper, M.D.	CALGB
Daniel Haller, M.D.	ECOG
Ben Sischy, M.D.	ECOG
Andrew T. Turrisi, M.D.	ECOG
Michael J. O'Connell, M.D.	NCCTG
James A. Martenson, M.D.	NCCTG
Robert J. Fitzgibbons, Jr., M.D.	NCCTG
Thomas D. Brown, M.D.	SWOG
Norman Estes, M.D.	SWOG
Jerry C. Rosenberg, M.D.	RTOG
Wun K. Hong, M.D.	RTOG
Muhyi Al-Sarraf, M.D.	RTOG
Arnold M. Herskovic, M.D.	RTOG
Valerie W. Rusch, M.D.	LCSG
Robert Ginsberg, M.D.	LCSG
David Kelsen, M.D.	MSKCC
Bruce D. Minsky, M.D.	MSKCC
Bernard Levin, M.D.	MDAH
Jack A. Roth, M.D.	MDAH
Bruce D. Cheson, M.D.	NCI
Timothy D. Moore, M.D.	NCI
Lawrence Rubinstein, Ph.D.	NCI
Elizabeth Anderson, Ph.D.	NCI
Harvey Pass, M.D.	NCI
Jay H. Hoofnagle, M.D.	NIDDK, NIH

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**TOPIC:** MULTIPLE MYELOMA  
**DATE:** February 21, 1989  
**COORDINATOR:** Bruce D. Cheson, M.D.

<u>Participants</u>	<u>Cooperative Group/Institution</u>
Barthel Barlogie, M.D.	MDAH
Daniel Bergsagel, M.D.	Ontario Cancer Institute
M. Robert Cooper, M.D.	CALGB
Robert A. Kyle, M.D.	ECOG
Malcolm R. MacKenzie, M.D.	SWOG
O. Ross McIntyre, M.D.	CALGB
Martin M. Oken, M.D.	ECOG
Sydney Salmon, M.D.	SWOG
Charles Schiffer, M.D.	CALGB

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**TOPIC:** ADVANCED OVARIAN EPITHELIAL CARCINOMA  
**DATE:** March 30, 1989  
**COORDINATOR:** Stacy R. Nerenstone, M.D.

<u>Participants</u>	<u>Cooperative Group/Institution</u>
Richard V. Smalley, M.D.	ECOG
Clarence E. Ehrlich, M.D.	GOG
William J. Hoskins, M.D.	GOG
George C. Lewis, Jr., M.D.	GOG
Maurie Markman, M.D.	GOG
William P. McGuire, M.D.	GOG
Tate J. Thigpen, M.D.	GOG
Stephen D. Williams, M.D.	GOG
David M. Gershenson, M.D.	MDAH
Samir Abu-Ghazleh, M.D.	NCCTG
John H. Edmonson, M.D.	NCCTG
Harry J. Long, III, M.D.	NCCTG
Karl Podratz, M.D., Ph.D.	NCCTG
David S. Alberts, M.D.	SWOG
Stephanie Green, Ph.D.	SWOG
Earl A. Surwit, M.D.	SWOG
Kenneth Swenerton, M.D.	NCI of Canada
Michael Christian, M.D.	NCI
Alison Martin, M.D.	NCI
Timothy D. Moore, M.D.	NCI
Eddie G. Reed, M.D.	NCI
Lawrence Rubinstein, Ph.D.	NCI
Giselle Sarosy, M.D.	NCI

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TOPIC: HEAD AND NECK CARCINOMA

DATE: April 17, 1989

COORDINATOR: Timothy D. Moore, M.D.

<u>Participants</u>	<u>Cooperative Group/Institution</u>
David J. Adelstein, M.D.	ECOG
Muhyi Al-Sarraf, M.D.	RTOG
John R. Clark, M.D.	DFCI
Mario A. Eisenberger, M.D.	UMCC
John F. Ensley, M.D.	SWOG
Arlene A. Forastiere, M.D.	Johns Hopkins Oncology Center
Karen K. Fu, M.D.	RTOG
Helmuth Goepfert, M.D.	RTOG
Wun K. Hong, M.D.	MDAH
John Robert Jacobs, M.D.	RTOG
Barbara Metch, Ph.D.	SWOG
Thomas F. Pajak, Ph.D.	RTOG
David E. Schuller, M.D.	Ohio State University
Todd H. Wasserman, M.D.	RTOG
Gregory T. Wolf, M.D.	VA Cooperative Group
Bruce D. Cheson, M.D.	NCI
Michael Christian, M.D.	NCI
Giselle Sarosy, M.D.	NCI
J. Michael Hamilton, M.D.	NCI
Michael A. Friedman, M.D.	NCI

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#### STRATEGY SESSIONS PLANNED FOR 1989

1. Myelodysplastic Syndromes--Standardization of definitions of response is warranted in MDS. This is of particular importance with the recent clinical availability of hematopoietic growth factors for MDS studies and the need for a focused approach.
2. Gastric Adjuvant--With the impending completion of the intergroup adjuvant study, future planning needs to begin.
3. Non-small Cell Lung Cancer--The exciting results with neoadjuvant therapy provide a stimulus for strategic planning for future studies in this tumor.
4. Malignant Melanoma (Adjuvant)--A strategy session will be planned for the Winter of 1989.

## LABORATORY-CLINICAL CORRELATIONS

Approximately 30 Phase III Group studies have formally described Lab-Clinical correlations. Some of the most interesting are:

1. Studies of the biologic significance of genetic rearrangements in the malignant cells of children with cancer offer increased reliability in correctly diagnosing patients and in predicting which patients are likely to relapse. Preliminary application of this methodology in the childhood neuroectodermal tumors has already demonstrated that (a) *n-myc* oncogene copy number is directly related to clinical outcome in neuroblastoma (CCSG), and (b) peripheral neuroepithelioma (PN), a tumor histopathologically indistinguishable from neuroblastoma, is in fact more closely related to Ewing's sarcoma; PN patients treated as Ewing's sarcoma do far better than those treated as neuroblastoma (M. Israel). These techniques will be applied to larger numbers of patients with solid tumors and leukemia. The cytogenetics data base being built by both pediatric cooperative groups will be available to identify patients whose tumor cells have rearrangements of specific gene regions of interest. Cells stored in group tissue banks or immunology reference laboratories will be used for studies of the relationship of immunophenotyping and cytogenetics in childhood ALL, and limited exploratory studies of the relationship between immunophenotyping and molecular genetics of childhood T-cell ALL. These efforts should provide a better understanding of the biology of ALL as it relates to overall response to the treatments offered.
2. Recent multivariate analyses of the CCSG data suggest that immunophenotype, using the cytoplasmic immunoglobulin marker and BA-1 (CD-24), adds to the prognostic importance of age and white blood cell count predicting outcome for ALL and displaces some of the prognostic and clinical markers thought to be quite important in the past. This and other important prognostic information supplied by cytogenetic analyses permits the tailoring of therapy to the particular risk that an individual child may have for a subsequent event or a successful remission induction. The conjecture that immunophenotype, early response data, FAB morphology and karyotype analysis will identify overlapping groups of patients with bad prognosis and that these patients may benefit from an early change in their therapy will be tested on the upcoming CCSG-1881 study.
3. SWOG and, more recently, ECOG myeloma studies are incorporating labeling indices and beta<sub>2</sub>-microglobulin determinations. Beta<sub>2</sub> microglobulin has been demonstrated in several successive SWOG studies to be the single best predictor of outcome.
4. SWOG and CALGB--a collaborative effort in AML and MDS to study phenotyping, cytogenetics, and sophisticated molecular biology studies with appropriate clinical correlations. Not only are all patients studied with currently available probes, but samples are being frozen which will be available to other scientists who propose a worthwhile scientific question.
5. Molecular studies and Deoxycytosine (DCF) pharmacology are now being incorporated into the large-scale hairy cell leukemia trials.

6. Clinical pharmacology studies are planned for the phase I DCF/fludarabine trials in CLL.
7. Based on the activity of both alpha- and gamma-IFN as single agents, trials are now ongoing in SWOG and CALGB which are evaluating the combination of these two agents in previously untreated CML patients. These studies also include state-of-the-art cytogenetics and molecular studies of the bcr gene using newly developed analytic techniques including polymerase chain reaction studies.
8. SWOG is performing studies in myeloma and non-Hodgkin's lymphoma which are exploiting the in vitro ability of verapamil to reverse MDR. In vitro correlates are an important part of the study.
9. Investigators in CALGB are evaluating the clinical importance of a number of newly identified phenotypic markers in CLL (e.g., CD5, shared idiotypes).
10. In CALGB's recently funded U10 application, support was provided for a clinical pharmacology program. One of the first drugs to undergo group-wide evaluation is amonafide. This is of particular importance since a high level of activity has been observed with this agent in previously untreated women with metastatic breast cancer.
11. CALGB is completing its study of ara-C pharmacology in patients with AML and attempts will be made to correlate blood levels with response.

#### FOREIGN INTERACTIONS

1. Dr. Ungerleider is a member of the International Society of Pediatrics (SIOP) and serves on that organization's subcommittee on treatment-related toxicities. In this capacity, he provides the Society with information collected by CTEP's Regulatory Affairs Branch regarding unexpected toxicities of anticancer agents used in children, for dissemination to the members of the Society, both in the USA and abroad.
2. US-USSR: CIB has developed with the Clinical Oncology Program, DCT a clinical trial in testicular cancer in the All University Cancer Center in Moscow. The trial compares cisplatin/VP-16 with CBDCA/VP-16 in limited stage testicular cancer.
3. US-India: CIB is coordinating development of an esophageal cancer trial with two cancer centers in India.
4. US-Japan: Dr. Friedman is the coordinator for the treatment area of this important scientific agreement for the exchange of research information.
5. US-Hungary: Dr. Friedman is the coordinator for this joint scientific agreement.
6. European Organization for the Treatment of Cancer (EORTC): Dr. Cheson is the liaison between the EORTC and CIB for clinical protocols.



7. National Cancer Institute of Canada (NCIC): Several CIB staff interact with Canadian investigators; Dr. Dorr is the official CIB liaison.
8. US-France: Dr. Cheson is the liaison between CIB and these investigators.
9. World Health Organization (WHO)/European Organization for the Treatment of Cancer (EORTC) Melanoma Activities: Dr. Nerenstone coordinates these activities with CIB.
10. US-Italy: Dr. Ungerleider is the liaison between CIB and Italian investigators.

## CONTRACTS

### Extramural Clinical Trials Office (ECTO)--EMMES

This contract provides operations and administrative support for a number of CTEP supported extramural research efforts. The services provided include: assistance in protocol and forms design; patient randomization; quality control data; coordination of scientific activities of clinical investigators, statisticians and project officers; planning of meetings and preparation of agenda, minutes, reports, communications, and related administrative tasks. The contractor also provides analytical support to CTEP in evaluating data obtained from extramural clinical research resources (such as the LAK-IL2 trials).

This contract also provides analytic support of the Intergroup Testicular Study, a collaboration among seven Cooperative Groups and four large institutions having an interest in testicular cancer. The protocol is a randomized controlled study of adjuvant chemotherapy of Stage II resectable testicular cancer and monitoring of Stage I testicular cancer.

For Stage II the study compares the disease-free and overall survival for surgery alone (with combination chemotherapy for relapse) versus surgery plus early adjuvant chemotherapy. Stage I patients are registered and monitored to identify prognostic variables which may predict recurrence in this group. The protocol also includes important biologic studies such as histologic typing, serum marker studies, and studies of the accuracy of lymphangiograms, CT scans, and ultrasonography. Progress presentations have been made at various Cooperative Group meetings: CALGB and SWOG. This study is nearing completion, and full analyses are forthcoming.

### MANAGING INVESTIGATOR-INITIATED GRANTS AND CONTRACTS (R Series, P01's, U01's, SBIR's)

The purpose of the CIB grants and contract management is to integrate relevant research information from all available sources, to disseminate the information contained in the grants and contracts to the Disease Coordinators of CIB and the Drug Monitors of IDB, and to serve as the primary contact for extramural investigators for administrative and scientific advice.

In FY 89, the CIB managed 126 investigator-initiated funded grants and contracts. These are broken down into the respective categories in the following table:

Code	Clinical Oncology	Cancer and Nutrition	Surgical Oncology	Total
R01	50	8	5	63
R13	4	0	1	5
R15	0	1	0	1
R29	4	2	0	6
R35	3	0	0	3
R37	2	0	1	3
R43	3	0	0	3
R44	3	0	1	4
<u>Subtotal</u>	69	11	8	88
P01	30	0	5	35
<u>Subtotal</u>	99	11	13	123
U01	1	0	0	1
<u>Subtotal</u>	100	11	13	124
SBIR Contracts	0	0	2	2
<u>GRAND TOTAL</u>	100	11	15	126

Most of the money in the grants/contracts pool was spent on P01 grants. CIB manages one of the largest portfolios of P01 grants both in terms of dollars and numbers of grants within the NCI. In FY 89, there was a net gain of 1 P01, increasing the total to 35. During FY 89, the Grants Program Directors attended 15 site visits for the review of P01's and performed 3 formal consultations on P01 submissions. During these formal consulting sessions the applicants bring drafts of their letters of intent, and the Grants Program Director along with other appropriate program staff (Disease Coordinators, Drug Monitors) give scientific as well as logistic advice. In addition to these formal consultations, numerous hours are spent on the telephone with both new and recompeting applicants. Approximately 57% of those P01 grants assigned to CIB were funded, a relatively successful rate.

These P01 grants serve as an important bridge between the preclinical and the clinical sciences. Many basic scientific advances are developed, refined and tested through the P01 grant mechanism and then developed into testable clinical hypotheses. The resultant clinical pilot studies in turn influence the basic science projects so that the desired synergistic effect is achieved. Several successful clinical pilot studies done in these P01's have become major studies in the Cooperative Groups. Thus, the P01 portfolio is an especially important and meaningful activity in CIB and represents the "cutting edge" of both basic and clinical research.

The number of active R01 grants managed by CIB has declined to a total of 63. There are several reasons for this decline: (1) a relative lack of grant submissions as compared to previous years (2) the transfer of 3 applications to other divisions of NCI due to a change in their research emphasis (3) the tough standards of grading by the ET I and ET II Study Sections. The success rate of obtaining funding in the R01 pool is very poor. Only 14% of those grants assigned to CIB are being funded. With the advent of percentiling during the third round of the review/funding cycle in FY88, the success rate of funding for grants assigned to CIB was projected to increase. This projected increase did not materialize. The Grants Program Director intends to continuously monitor the trend of funding for clinical research in FY 90 to determine if this downward funding rate is real and permanent. If this downward trend persists, steps will have to be taken to improve the grantsmanship of clinical investigators.

The SBIR grants and contracts continue to be an important component of the CIB program. Four phase I grants are funded at the present time and four SBIR grants have reached phase II. These include the creation of new software for access to cancer clinical trials and a database on questionable cancer therapies. A two wavelength laser for surgery is near completion. Anticancer, Inc. has developed an assay of chemotherapeutic responsiveness employing human tumor tissues growing in vitro on collagen gel matrices. One new SBIR contract was awarded during FY 89. This contract involves the development of a heat activated drug delivery system. Two new SBIR topics for contract solicitation have been approved for the coming fiscal year. One contract invites proposals for development of new assays to measure drug resistance of human tumors that may easily be adapted to mass screenings and have commercial potential. These may prove valuable in directing chemotherapeutic treatment of cancer patients. Contract proposals for the production of clinical grade, carrier-free radionucleotides and the radiolabeling of new investigational drugs for use in ongoing clinical trials will also be a new area of support.

CIB has been active in generating request for applications (RFA) to attract applicants into specific areas which need development or are ready for clinical study. During FY 89 a total of 4 RFA's were issued. CTEP recently issued an RFA on "Therapeutic Correlates of Drug Resistance" which invites grant applications involving the correlation of drug resistance to clinical response and development of clinical treatment to overcome drug resistance. Recent basic research efforts have uncovered numerous molecular and cellular mechanisms that may be operative in resistance to chemotherapy. We are encouraging investigators to develop clinical trials to correlate these measures of drug resistance with clinical response. Numerous number of letters of intent have been received. In collaboration with the Biological Response Modifiers Program, a RFA on studies of chronobiological effects in cancer treatment was issued. By understanding the differences in the circadian dependence of the response of normal and tumor cells to therapeutic agents, antitumor effects may be optimize while toxicity to normal tissues may be minimized during cancer therapy. Fourteen (14) applications have been received and are awaiting review. Twenty-four (24) research applications were received in response to the remaining two RFA's, 17 in the area of pancreatic cancer pain management (RFA #88-3) and 7 in pharmacodynamics of agents for bladder cancer intravesical therapy (RFA #88-4). Two grants in response to RFA #88-3 were funded and only one grant was deemed meritorious for funding in the latter RFA (#88-4).

### Highlights of Investigator Initiated Grants/Contracts

Several significant discoveries/leads with potentially important clinical applications/implications were made in FY89 by PI's who were supported by grants managed by CIB.

Dr. Joan Shapiro (R01 CA25956-10) has demonstrated heterogeneity in the karyotype and chemosensitivity of malignant human gliomas, defining one population of BCNU-resistant cells as near-diploid, with an over representation of chromosomes 7 and 22.

Dr. William Beck (R01 CA30101-09) found alterations in pst sites of the topoisomerase II gene from verapamil resistant CEM cells in studies aimed at characterization of "atypical" multidrug resistance expressed by human leukemia cells.

Dr. Richard Santen (P01 CA40011-01) is studying mitotic modifiers of hormone-dependent cancers. Studies using the Dunning R3327G prostate tumor model have shown that castration followed by testosterone priming followed by chemotherapy cause significant tumor regression when compared to castration plus chemotherapy. This cell kinetically based regimen is supported by kinetic studies showing manipulation of the S phase by castration and androgen priming. A series of experiments are ongoing to evaluate immunologic, pharmacokinetic, and nutritional aspects of these regimens.

Dr. William L. McGuire (P01 CA30195-08) and Dr. Gary Clark have completed a retrospective study of node-negative breast cancer patients demonstrating a positive correlation of diploid, low S-phase tumors status with disease-free survival (N. Angel. J. Med.). Studies on the expression of cathepsin D protein in breast cancer indicate that it is an important predictor of disease-free and overall survival for node-negative patients, but not for node-positive patients. Dr. McGuire's sub-project on the molecular mechanism of hormone resistance has uncovered a single, two-allele RFLP in the estrogen receptor gene, using the restriction enzyme *pvu* II. Analysis in 200 primary human breast tumor biopsies shows a significant, but not complete, correlation between absence of one allele and the failure to express receptor.

Dr. Douglas Tormey (P01 CA20432-12) has incorporated "maintenance endocrine ablation" into complex chemo-hormonal therapies for breast cancer with promising results. Results from protocols C0 7711 and C0 8214 utilizing intensive therapy with long-term tamoxifen demonstrate a complete remission rate of 77-85 percent, higher than any other study; and provide a 10 fold higher log tumor killing (0.86-1.26 log killing) than conventional therapy (0.15-0.18 log killing). These results have now been incorporated into ECOG trials.

Dr. Stuart Schlossman (5P01 CA34183-06) and his colleagues have developed a series of monoclonal antibodies reacting with AML and lymphoma cells. These antibodies are currently being used for leukemia and lymphoma cell purging in autologous bone marrow transplantation in NHL, ALL and AML. Using these antibodies Dr. Schlossman and his colleagues have developed a new class of immunotoxin conjugates. One such immunotoxin conjugate antibody is the anti-B4-blocked ricin immunoconjugate which is 3 logs more toxic for B lymphoma cells than is unconjugated blocked ricin. A phase I trial of this antibody has revealed limited toxicity with evidence for efficacy in both the response of

tumor and killing of circulating normal B cells. This preliminary data is very suggestive that anti-B4-blocked ricin antibody may modulate the B cell immune response. As such this antibody may be useful as an anti-human anti-mouse antibody reagent during immunoconjugate therapy.

Dr. Lawrence Einhorn (5R35 CA 39844-05) has 13 protocols that are pending review by the Institutional Review Board (2 in number) or accruing patients (11 in number). These protocols involve studies in germ cell tumors, small cell lung cancer, advanced breast cancer and prostate cancer. Studies performed in this grant period using Ifosfamide culminated in FDA approval of this drug in refractory testicular cancer. The studies to validate the concept of dose intensity in refractory germ cell tumors have provided the first convincing evidence that true cisplatin resistance can be overcome by the analog carboplatin and that the application of high dose chemotherapy with autologous bone marrow transplantation can be successful in highly-resistant solid tumors. The randomized prospective study on hormonally-resistant prostate cancer is near completion. This study evaluated the single agent cyclophosphamide vs the combination of cyclophosphamide + adriamycin + methotrexate and is the largest phase III study of its type in prostate cancer.

Dr. Emil Freireich (5R35 CA 39809-05) and his colleagues continue to identify the heterogeneity which exists in leukemia patients in remission by the detection of objective criteria of residual disease or objective markers of impending disease. Using sorted chromosomes obtained from Los Alamos Laboratories, they have been able to detect and characterize the non-random translocations associated with favorable prognosis in AML which include inversion 16, translocation 8;21 and translocation 15;17. Using the PCR technique they were able to detect residual disease in patients with CML and lymphoma (Blood in press, June issue, 1989). An important technical discovery was reported in this paper for amplifying the translocation 9;22, the Philadelphia chromosome. The translocation 14;18 was used as a marker for residual disease in treated patients with favorable histology nodular lymphoma and in intermediate grade lymphoma. Dr. Ann Killay in this grant has been able to produce evidence for the role of human chromosome 3 in murine fibrosarcoma tumor suppression and is currently investigating the involvement of this chromosome in the etiology of small cell lung cancer. She will also use techniques developed in this study to investigate the role of human chromosome 5 in human myelocytic leukemia.

Dr. Edward Neuwelt (5R01 CA 31770-07) reported a significant prolongation of survival of primary CNS lymphoma patients by combination chemotherapy given in association with osmotic blood brain barrier disruption. A 32 month median survival rate was obtained with 29 patients on the blood brain barrier disruption chemotherapy protocol as compared to a 13.5 month median survival recently reported in a series of 61 patients treated at Mass General Hospital. When sub-group analysis were performed on the 29 patients, it was determined that patients who entered this trial without receiving prior radiation therapy had an estimated long term disease-free remission rate of 65%, with no relapses beyond 12 months. These results await confirmation from other single institutional studies and from any future randomized trials.

Dr. Steven Grant (5 R01 CA 35601-07) has performed a series of phase I studies of deoxycytidine (dcyd) administered alone or deoxycytidine administered along with Ara-C in refractory leukemia patients. The rationale for this series of phase I studies was based on the laboratory observation that deoxycytidine preferentially protected normal versus leukemic progenitor cells from lethal actions of Ara-C. Dr. Grant has been able to give patients 22 gm/m<sup>2</sup> dcyd per day administered as a continuous 120 hr infusion along with an escalating dose of Ara-C (2,4 and 6.6 gm/m<sup>2</sup>) administered as a 96 hr continuous infusion and beginning 12 hr after initiation of the dcyd infusion. At these high doses of Ara-C there was minimal or no toxicity and all three patients experienced a decrease in the number and percentage of circulating blasts. In two of the three patients blasts were cleared from the peripheral blood, and bone marrow analysis revealed a partial response with a reduction in marrow blasts to 30%. These results potentially have far reaching implications in the use of Ara-C as an agent in the treatment of leukemias. Dr. Grant was invited to CTEP to present his data.

Dr. Ronald Hoffman (5R01 CA 34841-08) deduced the biological mechanism for the pathobiology of a clinical disorder of megakaryocyte (MK) production (Exp. Hemat. 16: 389, 1988). The patient had a 3.5 year history of a thrombocytopenic disorder characterized by 6-9 week cycles composed of 2-3 weeks of normal thrombopoiesis and 4-6 weeks of thrombocytopenia. The patient's serum was capable of almost completely blocking the ability of rGM-CSF, but not rIL-3, to promote MK colony formation and did not contain a cytotoxic antibody to the CFU-MK. This blocking activity was localized to an IgG fraction of the serum which blocked GM-CSF receptor function. The cyclical nature of the hematopoietic disorder was explained by perturbations of an anti-idiotypic antibody which neutralizes the action of the primary antibody.

Dr. Raymond Warrell (5R01 CA 42445-03) completed a randomized phase III study of gallium nitrate for treatment of bone loss in patients with multiple myeloma. 14 patients were accrued and all were evaluable. After their initial therapy for multiple myeloma, the patients were either observed for 6 months and then crossed over to receive gallium nitrate or were immediately treated with gallium nitrate for 6 months. The results indicated that the patients in the observation arm continued to lose bone mass while the patients treated with gallium nitrate had stable or some recovery of bone mass. The study did not demonstrate complete recalcification in gallium nitrate treated patients. However there was a major decrease in pain, and no episodes of hypercalcemia or cases of bone fracture. Patients on the control arm had one episode of hypercalcemia and 6 cases of bone fracture during the 6 month observation period. These findings strongly demonstrate the benefit of restoring bone mass by the therapeutic intervention with gallium nitrate.

Dr. James Speyer (5 R01 CA 3654-05) reported a positive clinical study in the New England Journal of Medicine 319:745, 1988 on the cardioprotective effect of ICRF 187 against adriamycin toxicity. The patients receiving ICRF 187 had less or little damage to the heart as compared to Adriamycin alone, determined by histopathological measurements from endomyocardial biopsies.

Dr. Charles Moertel (5 P01 CA 31224-05) reported his successful clinical trial of the regiment 5FU plus levamisole in Duke's C colon cancer patients to the National Cancer Advisory Board. This clinical result represents a major advancement in the treatment of colon cancer.

## Administrative Accomplishments

### Organ Systems Program:

The Director of CTEP and the Grants Program Director served as the Division representatives to the NCI Organ Systems Committee. The Organ Systems Program was reorganized during FY88-89. During FY88 the NCI Organ Systems Committee distributed all the grants (R01/P01) previously assigned to the Organ Systems Program to the different Divisions of the NCI according to program relevance/scientific content. Furthermore, this committee recommended that all new initiatives (RFA's, PA's) in a scientific area will be the responsibility of the Program Director assigned to that particular scientific area. During FY89, the Division representatives participated in making recommendations to the Executive Committee of NCI as to the location of the Organ Systems Program within the NCI structure and the future scientific role for the Organ Systems Program.

### Grantsmanship Seminars/Workshops:

The Grants Program Director participated in three seminars/workshops which offered guidance from a variety of perspectives to investigators who seek assistance grants for their research activities. The aim of these seminars/workshops is to offer a didactic explanation of the general organization, process, and functions of the grant process. These seminars/workshops were given to the Joint Section on Tumors for American Association of Neurological Surgeons and Congress of Neurological Surgeons; the Society of Chinese Bioscientist of America; and the American Association of Medical Informatics Congress 1989.

### Organization of Scientific Meetings:

The Grants Program Directors helped to organize two scientific meetings sponsored by DCT, drug resistance and Her 2/neu oncogene meetings. Our role in the drug resistance workshop was to provide a detail computer search of all grants funded by NCI and the American Cancer Society in the area of drug resistance. The workshop on oncogenes focused on two major areas of interest: the diagnostic/prognostic utility of Her-2/neu and its related oncogenes in human breast cancer and the therapeutic possibilities afforded by the biology of this gene. In the past few years several groups have shown a correlation between Her-2/neu or EGF receptor levels and clinical outcome in breast cancer patients. Representations from the Clinical Cooperative Groups were invited to participate in discussing with the basic researchers involved in these studies. A summary of the meeting will be submitted for publication as a meeting report to the JNCI. The Grants Program Director also participated in organizing a meeting on the human papilloma virus by providing suggestions on speakers and topics to be covered.

## SPECIFIC PROGRAM ACCOMPLISHMENTS

The Clinical Investigations Branch is oriented toward the clinical study of disease and/or modality issues. The following are selected highlights of the current program and specific plans for the future.

### PEDIATRICS

#### Accomplishments

1. A preliminary report of the Intergroup Rhabdomyosarcoma Study (IRS) - III was presented. This study enrolled 835 previously untreated patients from 1984-1988. Treatments consisted of 2 to 7 drugs in combination, radiotherapy in all but Group I favorable histology patients, second and third surgeries to document response and to excise residual disease, and intensification chemotherapy for partial responders. At 3 years, the overall survival rate of 73% in IRS-III is superior to IRS-II (67%), and IRS-I (60%), and the same relationship is true for CR duration. The major improvement is in patients with bulky residual disease but no metastases at diagnosis, which represent 53% of all patients entered (Proc ASCO 8:296, 1989).
2. The Children's Cancer Study Group has demonstrated that intensive systemic and intrathecal chemotherapy improves the outcome for infants with acute lymphoblastic leukemia. Infants less than 12 months of age represent the group of children with ALL at highest risk for both treatment failure and adverse sequelae of conventional CNS preventive therapy (cranial irradiation). In an attempt to improve outcome and to decrease long-term treatment effects, the CCGS utilized a multidrug regimen which included very high dose methotrexate infusions as part of CNS prophylaxis, but omitted cranial irradiation. The induction rate was 96% with this therapy, and life table estimate of the event-free survival at 3 years is  $48 \pm 5\%$ , representing a significant improvement over that of a recent historical control group ( $27 \pm 4\%$ ). The isolated CNS relapse rate was reduced from 20% to 5% without cranial irradiation (Proc ASCO 8:211, 1989).
3. The Pediatric Oncology Group reported preliminary evidence that the addition of radiotherapy to chemotherapy improves the outlook for children older than one year with non-metastatic neuroblastoma and positive (non-adherent to primary) intracavitary lymph nodes. Patients were randomized to chemotherapy alone versus chemotherapy plus irradiation to the primary tumor and regional nodes. Of the chemotherapy group, 50% achieved CR versus 77% of the chemotherapy plus radiation group; 31% of the former versus 58% of the latter remain disease free at a median of 38+ months.
4. The Children's Cancer Study Group and the Pediatric Oncology Group have embarked on a collaborative randomized trial evaluating the benefit of ifosfamide and etoposide when added to the standard therapy of newly diagnosed Ewing's sarcoma. Preliminary evidence indicates that the former agents are extremely active in this disease, at least in the setting of recurrent disease. The establishment of benefit, or lack thereof, of these agents when combined with vincristine, adriamycin, dactinomycin and cyclophosphamide, is the leading question regarding the optimum treatment of this disease.



## Future Plans

1. The Intergroup Rhabdomyosarcoma Study will open its fourth study in late 1989. IRS-IV will use a staging system independent of the extent of surgery for the first time (Proc ASCO 7:255, 1988). Stage III patients will be entered in a randomized study to determine the benefit of ifosfamide and etoposide when added to standard VAC therapy, as well as the benefit of hyperfractionated vs. conventional irradiation; the experimental arms are currently being piloted for feasibility. Newly diagnosed stage IV (metastatic) patients will be randomized to receive one of four drug pairs which are known to be active in previously treated patients prior to beginning conventional therapy. This design is intended to establish a rank order of activity for the drug pairs for use in future trials; additionally, active drug pairs will be integrated into the conventional therapy for individual responders.
2. Preliminary data from a study of very high dose methotrexate as a therapeutic option that permits the elimination of cranial irradiation in high risk patients with ALL has now been reported by the Children's Cancer Study Group (Proc ASCO 8:213, 1989) and has been incorporated in a recently activated randomized study (vs. BFM therapy).
3. The CCSG has launched a new protocol for acute non-lymphoblastic leukemia which includes an induction question and a comparison of three arms for post-induction therapy. For induction, 5 drugs are given simultaneously over 96 hours ("DCTER" regimen), and patients are randomly assigned to repetition of the drugs on days 10-13 irrespective of marrow cellularity vs. administration using cellularity on day 14 as the basis to decide when to repeat the cycle (the standard approach). The post-induction therapy consists of allogeneic transplant vs. autologous transplant after in vitro treatment with hydroxycyclophosphamide, vs. standard consolidation therapy.
4. Preliminary evidence indicates that transplantation of autologous bone marrow purged of malignant cells with magnetic immunobeads is an effective therapy for newly diagnosed advanced stage neuroblastoma in remission. The relative benefit of this toxic, complicated and costly procedure will be established by the Pediatric Oncology Group through a concurrently controlled clinical trial comparing transplant with conventional therapy.

## NODE NEGATIVE BREAST CANCER

Node negative breast cancer is diagnosed in 50,000 to 60,000 women each year. Three trials were made public in 1988 which demonstrated that the disease free survival rate associated with node negative breast cancer can be improved by the administration of adjuvant systemic therapy following primary surgical removal of the breast tumor with or without postoperative radiotherapy. These patients, heretofore, have been considered to enjoy such a good prognosis that adjuvant therapy has not been indicated. It is apparent, however, that 20-40% of these patients will develop recurrent metastatic breast cancer within 4 years of diagnosis and this recurrence rate can be decreased by 5% to 16% by either

chemotherapy or hormonal therapy (see table). The three studies were the NSABP B-13 and B-14 protocols and the Intergroup-0011 performed by SWOG, ECOG, and the CALGB. If the improved disease-free survival ultimately results in even a 5% survival improvement, then 2500 to 3000 lives may be saved by these treatments.

National Cancer Institute  
Summary of Recent Node Negative Adjuvant Studies

Disease Site	Type of Treatment Given	Disease-Free Survival and/or Survival (Improvement versus Control)	Number of Patients to Benefit if Treatment Widely Applied (Measured in Terms of Increased Survival/Additional Years of Life)
NSABP B-14 (Node Negative, Positive)	PLACEBO vs. TAMOXIFEN	83% vs. 77% (4 yr. DFS)	1500-2000 women/yr with prolonged disease-free survival 4 years after diagnosis
NSABP B-13 (Node Negative, Negative)	OBSERVATION vs. METHOTREXATE + 5-FLUOROURACIL	80% vs. 71% (4 yr. DFS)	1800-2400 women/yr with prolonged ER disease-free survival 4 years after diagnosis
INT-0011 (Node Negative, ER Negative, ER Positive if T $\geq$ 3 cm)	OBSERVATION vs. CYTOXAN, METHOTREXATE, 5-FLUOROURACIL	83% vs. 67% (4 yr. DFS)	7500 women/yr with prolonged DFS at 4 years

As part of the node negative studies, analysis of new prognostic factors are being evaluated retrospectively to see if patients at high risk of recurrence can be distinguished from those at a minimal risk of recurrence. Both DNA content and growth fractions are being examined. Preliminary results suggest that patients with tumors less than 2 cm and with normal DNA content and/or low growth fraction are at very low risk (<10%) of developing recurrent breast cancer in the first five years following diagnosis. These patients may perhaps be spared the cost and toxicity of treatments. This will be evaluated prospectively in the next generation of clinical trials.

At the time of the 1985 consensus conference for the adjuvant therapy of breast cancer, it was recommended that standard therapy for postmenopausal women with ER positive breast cancer should be tamoxifen outside of a clinical trial setting. The NSABP will soon report the results of their B-16 study which compared tamoxifen plus chemotherapy (PAF or AC). Disease-free survival has been significantly prolonged by the addition of either chemotherapeutic regimen to tamoxifen. No survival difference currently exists but this is the first adjuvant study which demonstrates a benefit for chemoendocrine therapy over endocrine therapy alone for this subset of patients.

## GU CANCER

### Prostate

In the past year the results of a study comparing leuprolide plus placebo with leuprolide plus flutamide in patients with newly diagnosed metastatic prostate cancer have been analyzed. Combination endocrine therapy produced a clinically and statistically significant prolonged survival compared to leuprolide plus placebo. On the basis of this study, the FDA has recently approved the use of flutamide in combination with leuprolide.

### Bladder

With the development of effective chemotherapy for transitional cell bladder cancer, CTEP organized an adjuvant trial in resectable locally advanced bladder cancer in which patients are randomized to radical cystectomy or to preoperative chemotherapy followed by MVAC. This study is one of the high priority trials and is now accruing well after an initial slow start. The study should be completed in 12-18 months.

## MELANOMA

### Accomplishments

1. Immune manipulation remains an area of active research in melanoma patients. Various cytokine combinations, either with cytotoxics (such as IL-2 plus cis-platin or DTIC), or with other biologicals (such as IL-2 plus alpha interferon and monoclonal antibodies) have continued to be tested in Phase II trials in advanced disease, attempting to optimize response while minimizing the severe toxicity seen with high doses of agents such as IL-2. ECOG is continuing a randomized Phase II study of gamma interferon in advanced disease patients in an attempt to find the most active dose of an agent which, in preclinical studies, may exhibit a bell-shaped dose-response curve. SWOG is continuing its adjuvant gamma-interferon study, while ECOG and NCCTG continues their alpha adjuvant trial.
2. Dose-intensity questions are being asked in patients with advanced melanoma using cytotoxic agents. High dose alkylating agents with autologous bone marrow transplantation has demonstrated significant activity in patients with metastatic disease, with metastatic CNS disease responding in some

patients. However, the response durations have been short. Researchers are continuing to investigate combinations of active agents in order to select agents that may have synergistic antitumor efficacy, including IL-2 followed by ABMT.

3. The usefulness of high dose cisplatin, with the chemoprotector WR 2721 is under investigation by ECOG in a Phase III trial in advanced patients. This remains an important clinical test of the hypothesis that more effective drug doses may be tolerated if a protective agent is administered. In order to combine this potentially useful combination with other known active agents, a Phase I pilot of CDDP with WR 2721 and IL-2 plus DTIC is actively accruing patients.
4. The importance of large surgical margins and prophylactic lymph node dissection in clinical Stage I patients continue to be addressed in the large Intergroup Melanoma Study. Hyperthermic isolation perfusion with L-PAM is also being studied by U.S. surgeons, in cooperation with the EORTC and WHO investigators, to prospectively evaluate the advantage of prophylactic perfusion in patients with intermediate thickness extremity melanoma.

#### Future Plans

1. Exploration of combinations of biologicals will continue, including the use of tumor infiltrating lymphocytes and monoclonals being tested in Phase I/II trials.
2. The use of CSF's will be evaluated using repeated courses of high dose therapy, in an attempt to duplicate the high response rates seen with the ABMT trials, but with the ability to repeat the cycles of therapy.
3. Phase II drug screening will continue in order to identify new, potentially active agents. A Phase I/II trial of L-PAM for isolated limb perfusion has been started this year, in an attempt to clearly define the perfusion MTD of this drug. It is hoped that the involved investigators will be able to continue Phase I and II drug testing of perfused drugs after the successful completion of this pilot study.

### GYNECOLOGIC MALIGNANCIES

#### OVARIAN CARCINOMA

##### Accomplishments

1. Phase III trials in advanced, suboptimal ovarian carcinoma continue to accrue patients. GOG is looking at two dose-intensities of CDDP combined with CYT. SWOG showed that the platinum analog CBDCA, is of equal efficacy as CDDP in a Phase III trial of CBDCA/CYT vs. CDDP/CYT.
2. The population of patients with advanced (St III), optimally debulked ovarian tumors is the focus of a Phase III intergroup effort, evaluating intraperitoneal CDDP with systemic CYT, versus intravenous CDDP/CYT. This

is a very important trial, involving SWOG and the GOG, given the theoretical advantage of regional therapy in small volume ovarian cancer, and is a large enough study to conclusively determine the role of upfront intraperitoneal therapy in this group of patients.

3. Patients who are NED after second look laparotomy are being enrolled in two separate studies of adjuvant intraperitoneal therapy. SWOG randomizes patients to alpha interferon versus observation, while the GOG is looking at p32.
4. Follow-up is now available for the GOG studies of early ovarian cancer. Patients with well and moderately differentiated St IAI and IBi tumors did well without any therapy. Patients with IC, II(A,B,C), and selected IAii and IBii tumors did equally well with L-PAM as with p32 therapy. Thus, standard treatment would be no further therapy for early patients, and p32 for patients with more advanced (but still localized) tumors outside a clinical trial.
5. Patients who fail standard treatment or recur after response to CDDP-containing regimens, require salvage therapy. Data from Johns Hopkins indicate taxol, a mitotic spindle poison, may have activity in this setting. Intraperitoneal single agents (such as mitoxantrone) and combinations (such as alpha interferon with CDDP) are actively being studied in the GOG, SWOG, and single institutions. Promising agents will be taken forward by the cooperative groups for definitive Phase III testing. Biological agents, such as tumor necrosis factor, and new cytotoxics, are also being studied.

Intraperitoneal 131I labeled B72.3 monoclonal antibody is being evaluated in Phase I studies in patients with refractory ovarian cancer, in collaboration with NCI investigators. Several other monoclonals will be ready for toxicity pilots in the near future.

#### Future Plans

1. For the treatment of patients who are NED after therapy CTEP will encourage the collaboration of SWOG and GOG in exploring useful therapies in this group of patients.
2. The positive study of taxol is being verified in a Phase II study by the GOG. If their results confirm the Hopkins' data, then the GOG plans a Phase III study combining CDDP with taxol versus CDDP/CYT. A third arm will be a combination of DCCP plus CBDCA attempting to increase the platinating activity of a regimen within the myelosuppression of the alkylator. Memorial Sloan-Kettering plans to study the drug when given intraperitoneally, as well, with the Phase II study to be carried out by the GOG.
3. Use of induction CBDCA, followed by CDDP therapy, is the sequential approach to increasing dose intensity that SWOG will pilot in advanced ovarian patients.

4. Pilot studies of ABMT in patients with relapsed ovarian cancer are being piloted in several cancer centers. This approach will be expanded to several GOG member institutions using a combination of ifosfamide/CBDCA because ifosfamide has shown activity in relapsed patients. In the future, CSF's will be added to decrease myelosuppression, thereby avoiding the need for bone marrow infusion.

#### CERVICAL

1. The Cooperative Groups have continued extensive Phase II screening of drugs in this disease. The GOG has demonstrated a 40% objective response rate with Dibromodulcitol in patients with advanced or metastatic disease.
2. Locally advanced patients (St IIB, III, and IVA) continue to be entered into a GOG study (which SWOG has just joined), comparing the radiosensitizing effect of Hydroxyurea versus a combination of CDDP-5FU.

#### Future Plans

1. The positive DBD study will be the basis for a randomized Phase III trial, with CDDP versus CDDP-DBD combination. Response rate, and duration of response will be the endpoints of interest. As bone marrow suppression, particularly thrombocytopenia, was the major toxicity encountered in these patients, a future study with escalating doses of DBD using CSF's would be of interest.
2. An adjuvant study of 5FU + bolus CDDP after radiation therapy for selected early patients (St IA<sub>2</sub>, IB, and IIA) is under discussion as an Intergroup study with the GOG and SWOG. Since this is a relatively small subset of patients, the feasibility of adequate accrual has not yet been fully explored.
3. Novel radiation fractionation schemas will be tested by RTOG and GOG in an attempt to increase local control in advanced cervical cancer patients.

#### SARCOMAS

##### Accomplishments

1. Accrual to an intergroup osteosarcoma trial comparing adjuvant post-operative therapy with 3 (HDMTX-DOX-CDDP) versus 6 (same + BLEO-ACTD-CTX) drugs has been slow. In addition, unexpected toxicity has been seen on the 3 drug arm, possibly related to the short interval between the administration of CDDP and high dose MTX, which may be dropped from the regimen.
2. The Phase III trial of doxorubicin with DTIC ± Ifosfamide in advanced soft tissue sarcomas continues to rapidly accrue patients. A new pilot trial using GM-CSF will attempt to dose escalate this regimen in hopes of increasing the response rate and response duration.

3. ECOG continues accruing on its advanced sarcoma protocol of 3 regimens: single agent doxorubicin, doxorubicin + Ifosfamide, and doxorubicin + mitomycin C + cisplatin.
4. The Intergroup Sarcoma Committee is using the results of material collected for pathologic review in subsequent analysis of data, in an attempt to advance the clinico-pathologic understanding of these tumors. Basic biological questions will also be addressed, such as oncogene expression and multidrug resistance, using fresh tumor specimens.
5. Confirmatory studies of IUDR as a radiosensitizer are being undertaken by cooperative group investigators, based on the preliminary work of radiation oncologists at the NCI. A Phase III study will follow if the initially positive results are confirmed.
6. An Intergroup Sarcoma Trial of adjuvant therapy of soft tissue sarcomas is accruing patients using doxorubicin, DTIC, and Ifosfamide.

## BREAST CANCER

### Accomplishments

1. In the past year, three NCI-funded randomized clinical trials in node negative breast cancer were analyzed with each showing an advantage as measured by disease-free survival for the treated patients compared to those receiving no treatment. The results of these three studies were made public through the use of a Clinical Alert. In NSABP B-13 node-negative patients with estrogen receptor negative tumors were treated with methotrexate and 5-fluorouracil or were simply observed. At 4 years treated patients had an 80% chance of being disease free while untreated patients had only a 71% chance of being free of recurrence, a difference that was highly statistically significant ( $p=0.003$ ). This therapeutic benefit was observed for patients  $\leq 49$  and  $\geq 50$  years of age. No difference in survival has yet been observed.

In NSABP B-14, estrogen receptor positive, node-negative patients were randomized to tamoxifen or to placebo. There was a statistically significant difference in disease-free survival favoring the tamoxifen treated patients ( $p<0.00001$ ). This benefit also was found in patients  $\leq 49$  and  $\geq 50$  years of age. Again, no overall survival differences have been identified at this time.

In the Intergroup study conducted by ECOG, SWOG and CALGB, patients with node negative breast cancer were randomized to either CMFP or observation. Eligibility included all estrogen receptor negative tumors as well as estrogen receptor positive tumors greater than 3 cm. A disease-free survival advantage was identified for the treatment arm of this study which when analyzed by subsets was present for premenopausal and postmenopausal patients as well as for ER positive and ER negative patients. No survival advantage has been identified as of yet.

## Future Plans

1. A study of preoperative versus postoperative chemotherapy has recently been started by the NSABP for Stages I and II disease.
2. Several pilot studies attempting to intensify breast cancer chemotherapy by employing colony stimulating factors are being developed to identify a regimen which can be safely studied in the adjuvant setting in order to rigorously test the concept of dose-intensity.
3. In node negative breast cancer, the role of sequential chemotherapy and hormonal therapy is to be tested in estrogen receptor positive patients. In estrogen receptor negative patients, duration of chemotherapy as well as the relative benefit of doxorubicin versus non-doxorubicin containing regimens will be tested.
4. Several different high-dose chemotherapy regimens with autologous bone marrow support are being developed with and without colony stimulating factors.

## UROLOGIC CANCER

### Accomplishments

1. During the past year, the prostate intergroup study has matured and has identified a modest but statistically significant survival advantage for combined leuprolide + flutamide compared to leuprolide alone. Further follow-up will better define the extent of this survival advantage.
2. A study to test the benefit of preoperative chemotherapy using the MVAC regimen in locally advanced bladder cancer has been started during the past year. The study is designed to give three courses of MVAC followed by radical cystectomy versus radical cystectomy alone. The study was initiated by SWOG with ECOG also involved in the study's development. In addition it has been designated a high priority trial by the NCI Board of Scientific Counselors.
3. Several studies in early stage prostate cancer have been developed during the past year including the role of adjuvant hormonal therapy for Stage D<sub>1</sub> disease, the role of adjuvant radiation in Stage C disease with positive margins following radical prostatectomy, and the relative merit of radical prostatectomy or radiation for Stages A<sub>2</sub> and B disease (not yet started).
4. The population of patients with poor risk germ cell tumors has been further defined by investigators at Memorial Sloan-Kettering Institute. Additionally, the less toxic combination of cisplatin and VP-16 has produced equivalent results to VAB-6 in an early analysis by the same investigators.



5. An intergroup study of RT versus BEP chemotherapy in patients with advanced Stage II testicular seminoma has recently been started and will take three to five years to complete. This study follows important leads suggesting that chemotherapy reduces distant recurrence compared to the standard of treatment, radiotherapy.

### Future Directions

1. Biologic therapies in renal cell cancer are being further developed including tumor infiltrating lymphocytes  $\pm$  IL-2, tumor vaccines, and LAK + IL-2.
2. A direct comparison of BCG with mitomycin-C in superficial bladder cancer will be conducted by SWOG.
3. Efforts to limit the toxicity of therapy for good risk germ cell cancer are being conducted in separate studies by MSKCC (with SWOG) and by ECOG. The MSKCC trial compares VP-16 + cisplatin with VP-16 + carboplatin. The ECOG study will compare VP-16 + cisplatin with or without bleomycin.

### NEUROENDOCRINE TUMORS

1. Pilot studies continue in advanced patients, including a randomized Phase II study of alpha interferon and doxorubicin versus VP-16 and cisplatin.
2. A Phase III intergroup study will soon be opened, randomizing advanced islet cell patients between chlorozotocin + doxorubicin versus streptozotocin + doxorubicin versus a phase II drug. On relapse, refractory patients would receive one of the doublets. This would allow the testing of new agents in unpretreated patients, in order to maximize the likelihood of finding antitumor effects. The initial phase II drug may be pibenzimol, a drug found to induce pancreatic necrosis and cause diabetes mellitus during phase I studies.

### ADULT HEMATOLOGIC MALIGNANCIES (LEUKEMIA AND LYMPHOMA)

#### ADULT LEUKEMIA

##### Accomplishments

1. A number of clinically important subsets of adult acute lymphoblastic leukemia (ALL) patients have been identified by the CALGB using newly developed monoclonal antibodies. In approximately 30% of morphologically and histochemically diagnosed ALL, the immunologic phenotype is ambiguous. Patients whose blasts carry myeloid antigens have a particularly poor prognosis. This may provide the basis for phenotype directed clinical trials.

2. In a carefully conducted, prospective randomized trial, ECOG has demonstrated the need for post-remission therapy in adult ANLL in a 3-arm study which compares no further therapy (arm closed early) with maintenance vs intensification. The comparison of the latter two arms demonstrated an advantage to a single course of intensification over prolonged maintenance.
3. CLL trials are underway exploring various combinations of DCF and fludarabine with either conventional agents or with themselves. A national phase III trial will eventuate.
4. Both alpha- and gamma-IFN have been shown to be active in patients with chronic phase CML. CALGB and SWOG are currently exploring the tolerability of various schedules of combinations of these agents in previously untreated patients. An eventual phase III trial will be developed, although the "standard" therapy arm remains to be determined.

#### ADULT MALIGNANT LYMPHOMA

##### Accomplishments

1. ECOG, CALGB, and SWOG are collaborating in an important comparison of MOPP/ABVD with the newly described MOPP/ABVD hybrid in previously untreated Hodgkin's Disease. Accrual is more rapid than projected.
2. Patients with advanced stage, low-grade, or indolent NHL have been considered incurable with conventional chemotherapy, with or without radiation. Unfortunately, these patients have rarely been treated aggressively with one of the third generation multi-agent regimens. SWOG is now treating such patients with the intensive ProMACE/MOPP program, randomizing responders to alpha-interferon or observation alone.

##### Future Plans

CALGB is developing an intensive CHOPE regimen which will require G-CSF with the eventual comparison of CHOPE at its MTD vs CHOP/CSF at that MTD.

#### BONE MARROW TRANSPLANTATION

##### Accomplishments and Future Plans

1. Using high dose chemotherapy with combinations of alkylators requiring autologous marrow rescue, investigators from the Dana-Farber Cancer Institute have reported exciting response rates in heavily pre-treated patients. For example, 90% of women with metastatic breast cancer achieve a response, 10-20% of which are CR's. Future directions include substitution of analogues to reduce toxicity and increase efficacy, and to develop disease-directed combinations in definitive studies.

2. ABMT in Stage III breast. A multi-center trial is ongoing in CALGB to test high-dose, multi-agent chemotherapy with marrow transplant in women with stage III breast cancer. Traditionally most of these women relapse and die after conventional chemotherapy/and or radiation therapy with or without surgery. This protocol offers an innovative approach for those high risk patients.
3. SWOG and ECOG have each recently activated an important prospective, randomized comparison of allogeneic BMT, ABMT, and conventional consolidation chemotherapy in patients with AML in first CR.
4. ECOG is conducting a pilot study of MACOP-B followed by autologous marrow transplantation as front-line therapy for patients with intermediate and high-grade NHL with poor prognostic features. Such risk-directed strategies are an important advance in our approach to therapy.

#### ADULT MALIGNANT LYMPHOMA

It is becoming increasingly apparent that there are a number of molecular and cytogenetic factors which may have important prognostic relevance in NHL. For example, the bcl-2 oncogene has been cloned from patients with follicular lymphomas. These molecular studies have, to date, only been evaluated in a limited number of cases. With current technologies, CALGB plans to perform molecular genetic studies on larger numbers of patients, correlating these findings with response to treatment and survival.

#### MALIGNANT BRAIN TUMORS

##### Accomplishments

1. Interstitial Versus External Beam Radiation Therapy--Gliomas Interstitial irradiation for malignant tumors in the brain has been administered widely throughout the country. From center to center the isotopes, surgical techniques, and dosimetry are high variable. The BTSG currently has a Phase III comparison of external beam versus external beam plus interstitial irradiation for malignant gliomas.

Efforts to improve the effectiveness of cranial irradiation through hyperfractionation or halopyrimidine radiosensitization are undergoing study in the RTOG. Other Cooperative Groups and Cancer Centers continue to explore phase II chemotherapeutic agents in malignant gliomas.

##### Future Plans

1. The current standard treatment of primary CNS lymphoma is surgery and/or radiation therapy. Several group studies are testing multiagent chemotherapy with radiation. Patients eligibility will not include patients with AIDS.

## GASTROINTESTINAL CANCERS

### ACCOMPLISHMENTS

#### Esophageal Cancer

Phase III comparisons in the RTOG and ECOG for localized esophageal cancer tested radiation alone with radiation plus cisplatin based chemotherapy. The ECOG study is complete and shows a benefit for the combined modality arm. The RTOG study will complete accrual in the next few months.

#### Colorectal Cancer

##### 1. NSABP Colon C02 and NCCTG 79-46-04

Phase III adjuvant trial of postoperative, seven day, 5-FU portal vein infusion versus surgery alone have been completed in both NSABP AND NCCTG. The NCCTG trial was reported in May 1989 as showing no benefit for adjuvant portal vein 5-FU. The NSABP trial is still being evaluated.

##### 2. NSABP C03 and C04

Adjuvant trial for colon cancer activated by the NSABP in 1987 employing the previous best chemotherapy arm of MOF (MeCCNU, Vincristine, 5-FU) randomized against chemotherapy with 5-FU/leucovorin. Very rapid accrual to this trial permitted closure with an 1800 patient sample in April 1989. A replacement comparing 5-FU/leucovorin/levamisole versus 5-FU/leucovorin versus 5-FU levamisole was activated in June 1989.

##### 3. NCCTG Rectal Trial (86-47-51)

The current NCCTG rectal adjuvant trial employs combined radiation + chemotherapy and uses a 2x2 factorial design. It will evaluate the contribution of MeCCNU to 5-FU for response and toxicity, compared to 5-FU alone. It will also evaluate the benefit of continuous infusion 5-FU during radiation therapy compared to intermittent bolus 5-FU. High priority status has been assigned to this trial and has stimulated accrual.

##### 4. IG Colon Adjuvant Trial

Based on the NCCTG experience with the role of the immune modulator, levamisole, a confirmatory intergroup Phase III trial in adjuvant treatment of colon cancer was started in 1985. Accrual of 1200 patients was accomplished in 2.5 years. The results are expected within the next year.

##### 5. NCCTG (87-46-51) and Intergroup (INT 0089) Colon Adjuvant Trials

Four Cooperative Groups (NCCTG, ECOG, SWOG, CALGB) are comparing 5-FU/leucovorin versus surgery alone for adjuvant therapy of colon cancer. Accrual is progressing rapidly with completion expected within one year.

## FUTURE PLANS

1. **ESOPHAGEAL CANCER:** Two Intergroup protocols were generated from the Esophageal Strategy Meeting (January 1989) and are expected to be activated in 1989. The first protocol will compare surgery alone with surgery plus chemotherapy for patients with completely resected tumors. For patients with local regional disease, the earlier trials from ECOG and RTOG (see above) will be built upon with a comparison of neoadjuvant chemotherapy followed by chemo/radiation versus chemoradiation alone.
2. **GASTRIC CANCER:** An intergroup gastric adjuvant protocol will be activated in 1989 testing 5-FU/leucovorin + radiation versus surgery alone.
3. **COLON CANCER:** The NCCTG will organize an intergroup trial with SWOG and ECOG to test the efficacy and toxicity of streptozotocin plus adriamycin versus chlorozotocin plus adriamycin versus new agents (e.g., interferon, pibenzimol) for advanced islet cell tumors.

## LUNG CANCER

### NON-SMALL CELL

#### Accomplishments

1. The CALGB reported early closure of a positive trial showing the survival benefit of induction Cisplatin and vinblastine before radiation for locally advanced but unresectable non-small cell lung cancer. (NSCLC). They are currently building on this study with several pilot trials investigating the relative efficacy of simultaneous chemo-radiation therapy and the role of carboplatin. The more promising pilot regimen will be compared to sequential chemo-radiation therapy in their next phase III study.
2. Based on the CALGB experience in locally advanced but unresectable non-small lung cancer, RTOG is currently conducting a confirmatory study in which a third arm has been added. This will look at the effectiveness of hyperfractionated radiation therapy, which a previous RTOG study indicated prolonged survival in this patient subset.
3. The NCCTG has recently initiated a phase III adjuvant study in patients with completely resected stage III-A non-small lung cancer. These patients currently are at high risk of early relapse. This ambitious trial will investigate the role of chemotherapy, radiation therapy, or chemo-radiation therapy versus an untreated control group. A similar study is also being planned by the RTOG.
4. Improvements in radiotherapy are an active area of investigation, including alterations in fractionation (high doses once weekly, low doses several times daily), combinations with radiosensitizing drugs (SR-2508, WR-2721, misonidazole), intraoperative brachytherapy, and neutrons rather than conventionally used photons. The RTOG, CALGB, and ECOG will be exploring these questions in several clinical trials.

5. An enhanced interrelationship between the NCI epidemiologic program and CIB has been developed. An epidemiology developed protocol administering debrisoquine to assess drug (and possibly carcinogen) metabolic rates has been adopted by the LCSG.

## SMALL-CELL

### Accomplishments

1. In limited stage small cell lung cancer ECOG has taken a regimen which they piloted incorporating Cisplatin VP-16, and concurrent accelerated-hyperfractionated radiation therapy into phase III testing. The median duration of survival has not been reached at 2 years in the limited institution pilot study, which compares favorably with previous results (10-16 month median survival). RTOG will also participate in the phase III trial.
2. SWOG has recently initiated a toxicity amelioration study in limited small cell lung cancer. It will assess the effectiveness of GM-CSF in ameliorating the toxicity associated with concurrent chemo-radiation therapy (Cisplatin/VP-16). Responders to induction therapy will then be re-randomized to either alpha-interferon or observation. This will build upon preliminary results from Europe the effectiveness of interferon as maintenance therapy in small cell lung cancer.

## HEAD AND NECK CANCER

### Accomplishment

1. The head and neck intergroup trial compares postoperative radiation with postoperative radiation plus cisplatin-5-FU in the adjuvant setting (pathologically negative margins). The trial had encountered difficulties with patient compliance on the combined modality arm; however by changing the randomization to the postoperative setting, accrual has increased substantially. Accrual goals should be reached by July of this year.
2. The Head and Neck Intergroup has recently initiated a phase III trial in nasopharyngeal carcinoma comparing simultaneous Cisplatin/RT followed by maintenance Cisplatin/5-FU to radiation alone.

### Future Directions

1. The simultaneous use of Cisplatin and radiation therapy may be incorporated into proposed intergroup trials. WR-2721 will be studied in the recurrent disease population for its ability to protect the patient from the toxicities associated with chemotherapy and radiation therapy. Finally the Head and Neck Intergroup is considering a functional preservation trial in patients with advanced disease in the larynx to confirm the results recently reported by the Veterans Administration Cooperative Study Group.

## ANTICIPATED ACTIVITIES (FY90) FOR THE CLINICAL INVESTIGATIONS BRANCH

The challenge for the upcoming year will be to maintain CIB's current activities while expanding into new areas during a period of serious fiscal constraints. A new generation of high priority trials will be inaugurated; attention will be paid toward increasing the participation of minority patients in the clinical trials process, improving upon the striking increased cancer mortality seen among Blacks, native Americans, native Hawaiians and other minorities; new clinical trials will be sought in those diseases with a disproportionate incidence and mortality in minorities (e.g., multiple myeloma, esophageal carcinoma; inappropriate obstacles to the participation of the elderly in clinical trials will be removed; and the Clinical Trials Cooperative Groups will be encouraged to become a resource for the entire National Cancer Institute for the conduct of trials in cancer prevention, etiology, and biology.

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## INVESTIGATIONAL DRUG BRANCH

### 1. Description

The Investigational Drug Branch (IDB) is responsible for sponsoring trials of new investigational drugs and evaluating them for efficacy and toxicity. It does this by: (1) coordinating and monitoring the trials of new agents developed by the DCT; (2) planning with members of the Clinical Investigations Branch overall strategies for new agent studies in specific tumor types; (3) regulating the distribution of investigational new drugs for which DCT is the sponsor; (4) maintaining close contact and ongoing dialogue with the pharmaceutical industry in an attempt to ensure that new agent development proceeds in a coordinated way.

The Investigational Drug Branch is divided into three sections: two medical sections, one for cytotoxic agents and one for biologic response modifiers, which are concerned with the clinical aspects of the drug development process, and the Drug Management and Authorization Section, which regulates the distribution of investigational new drugs to all NCI-sponsored investigators. The professional staff of the Branch includes 9 physicians, 4 pharmacists, 1 Ph.D. and 1 registered nurse.

### 2. Accomplishments

#### a. Clinical Trials with Investigational Agents

##### 1) Alpha Interferon

Although licensed in this country for Hairy Cell Leukemia and Kaposi's Sarcoma, investigational studies in a variety of disease types are still being pursued. Adjuvant studies in renal cell carcinoma, melanoma and ovarian cancer are still ongoing.

Preclinical studies have indicated that IL-2 and alpha IFN have synergistic antitumor activity when administered together and a recently completed Phase I trial at the NCI documented antitumor activity in patients with renal cell carcinoma and malignant melanoma. Recently, a high response rate was observed in previously untreated patients with advanced colorectal carcinoma who received 5-FU in combination with alpha IFN. The results of these studies are currently being confirmed.

Recently interferon-alpha in combination with 5-fluorouracil showed promising activity in patients with untreated metastatic colorectal cancer. Confirmatory studies have been initiated, and the activity of this combination in other GI malignancies are being performed under the CTEP phase II/III contract.

## 2) Gamma Interferon

Previous clinical trials conducted by the NCI identified a dose and schedule for gamma interferon administration which resulted in optimal biological activity. Large scale adjuvant trials which use this regimen of gamma interferon administration are currently in progress in malignant melanoma, colon and small cell carcinoma of the lung. Two of these trials are expected to complete accrual by the end of this year, although follow-up for response and survival will continue. Monocyte function of patients treated on these studies is also being measured and will be correlated with antitumor activity. A recent Phase II study has revealed that 3 SCLC patients who were partial responders to PACE chemotherapy converted to CR status following IFN-gamma administration. Two Phase II trials of IFN-alpha + IFN-gamma in CML are ongoing as is a trial of ex vivo treatment of bone marrow with IFN-gamma during bone marrow transplantation of CML patients.

## 3) IL-2

IL-2 alone, using a high dose bolus schedule, produced complete and partial responses in 10/47 (21%) melanoma patients. Phase II trials in a number of disease sites (renal, colon, lymphoma, breast, pancreas, lung) have opened using a less toxic continuous infusion schedule of IL-2. However accrual to these studies is threatened by inability of investigators to receive reimbursement from insurance companies for prolonged hospital stays. Pediatric Phase II trials using a similar schedule are set to open.

## 4) IL-2/LAK

1. Patients with melanoma or renal cell cancer who have obtained complete remissions, following IL-2/LAK remain in unmaintained remission at 12-36 + months following treatment. Approximately 5% of patients achieve complete remissions with treatment.
2. A randomized study in renal cell cancer patients comparing continuous infusion versus bolus administration of IL-2 in combination with LAK cells found no significant difference in response or toxicity. Response rates were approximately 20% in both arms. However successive Phase II studies demonstrated that continuous infusion IL-2 with LAK cells was ineffective in melanoma patients, while bolus administration of IL-2 with or without LAK produced response rates of 20-25%.

The Modified Group C program has been safely initiated at 14 NCI Clinical and Comprehensive Cancer Centers and has been accruing 10 patients per month. The Modified Group C randomized study of IL-2 versus IL-2/LAK, to determine if LAK contributes to the efficacy of IL-2, continues to accrue patients. Rosenberg et al will publish the results of a similar randomized trial, which showed no statistical differences between arms but suggested an increased complete response rate when LAK were administered with IL-2. Ongoing BRMP trials are studying means of generating effector LAK cells of greater potency.

#### 5) IL-2/TIL

The Surgery Branch published their results with IL-2/TIL therapy. Although the reported response rates are quite high in melanoma (11/20), this therapy is limited by the ability to obtain tumor from patients and expand the infiltrating lymphocytes into a large number of highly specific tumor effector cells. The NCI is sponsoring TIL trials with extensive basic science corollary laboratory studies to further define the properties of these cells. Other methods of generating antigen specific cells are being pursued, including the use of in vivo active specific immunotherapy (vaccination with autologous tumor) followed by further ex vivo expansion of these antigen primed cells with autologous tumor. In animal models this method has resulted in generation of antigen specific cells even from non-immunogenic tumors.

In the past year several trials of active specific immunotherapy in combination with IL-2 have been approved. In melanoma both autologous and allogeneic tumor cell vaccines are being explored in combination with IL-2 in patients with advanced disease. A similar study in metastatic renal cell cancer using an autologous tumor vaccine has begun.

Trials of IL-2 in combination with chemotherapy are proceeding. High dose IL-2 followed in sequence by high dose cis-platinum produced a 40% response rate, but with significant toxicity. Other schedules combining cis-platinum and IL-2 are under investigation. Two trials of adriamycin with IL-2 are ongoing. Based on marked preclinical synergy and initial reports of clinical antitumor activity the NCI has sponsored combination trials with cyclophosphamide and IL-2. The results on these Phase II studies are pending. Several trials of combination chemotherapy with IL-2 in melanoma have been initiated in hopes that combining two active modalities will lead to overall improved response rates and response durations.

Other innovative trials involve the use of IL-2 as a marrow and immune cell protectant when administered with high dose chemotherapy. Based on elegant preclinical models the first study of high dose chemotherapy, followed by autologous marrow incubated in IL-2 and returned to the patient, was approved. In the animal models effector cells could be generated in the marrow which mediated antitumor effects when infused into the animal and combined with systemic IL-2 administration.

Adjuvant studies have also been considered and approved. A cooperative group plans to administer systemic IL-2 to patients with acute myelogenous leukemia in second complete remission. The toxicity of high dose regimens has hindered attempts to perform adjuvant trials in diseases where IL-2 has been active in the advanced setting.

Trials employing alternate routes of administration are in progress including intratumor, intraperitoneal, hepatic and splenic intraarterial, and intracavitary brain.

#### 6) Tumor Necrosis Factor

Broad Phase II testing continues to define the antitumor activity of TNF when given as a single agent. Other specific areas of interest that are being pursued include: combination studies with topoisomerase II inhibitors or IL-2 based on preclinical evidence of synergistic antitumor activity; pediatric Phase I trials of TNF alone or with a topoisomerase II agent; and Phase I trial of intravesical TNF for bladder cancer.

#### 7) Combination Cytokine Regimens

IL-2 in combination with interferon-alpha appears to be a promising regimen. Initial trials indicate increased response rates in melanoma and perhaps renal cell cancer. In renal cell cancer lower doses of these agents, which can be administered in the outpatient setting, may be active. Phase II trials are in progress, and high dose comparative trials in renal cell carcinoma and melanoma (IL-2 versus IL-2/IFN) are set to begin in the IL-2/LAK contract centers.

Phase I trials of TNF followed in sequence by IL-2 are ongoing. A Phase I trial of concurrent administration of these agents was completed, and antitumor activity was noted in non-small-cell lung cancer. Phase II trials in a limited number of disease sites are being solicited.

Phase I trials of IL-2 in combination with interferon gamma are near completion. These trials will be reviewed and the most active schedules (immune enhancement and antitumor activity) will be explored further in Phase II trials.

8) Colony Stimulating Factors

Early studies have also shown that G-CSF and GM-CSF can reduce the neutropenia associated with chemotherapy, bone marrow transplantation, and some congenital or acquired hematopoietic deficiency states. To reduce toxicity or to permit administration of more treatment, the NCI is adding CSFs to active chemotherapy regimens used for the treatment of breast cancer, lung cancer, ovarian cancer, multiple myeloma, lymphoma, testicular carcinoma, and sarcomas. CSFs are also being tested in combination with bone marrow transplantation to shorten the time to hematopoietic reconstitution as well as to augment harvest of progenitor cells from the peripheral blood. In addition, CSF's are being used as growth factors in patients with relapsed/refractory leukemia in an attempt to recruit quiescent malignant cells in cell cycle to increase responses to standard cell-cycle specific therapy.

9) Levamisole

The results of a recent trial from the NCCTG using 5-FU + levamisole as adjuvant therapy for resected colon cancer suggested that this combination may prolong the disease-free and overall survival of patients with Dukes' C disease. This observation is being studied in a confirmatory Intergroup trial; the NCI and FDA have designated 5-FU/levamisole as a Group C/Treatment IND.

10) Monoclonal Antibodies (MoABs)

A protocol to be used at NCI contract institutions has been developed to permit rapid comparison of multiple MoABs directed against the same antigen. This approach will be used to determine the optimal variable region for future MoAb constructs and will initially study MoABs directed against the CEA and TAG-72 antigen in patients with colorectal carcinoma. An anti-CD3 MoAb will be assessed for enhancement of T-cell function in patients with solid malignancies. Protocols have been initiated to evaluate MoAb-toxin conjugates. An anti-CD5 MoAb conjugated to ricin A chain, previously used to deplete T-cell populations in patients with CD5 (+) neoplasms. In addition, an anti-ovarian carcinoma MoAb conjugated to pseudomonas exotoxin is undergoing clinical evaluation. Studies to further define the activity of the anti-GD3 MoAb, R24 in malignant melanoma are currently in progress. The clinical evaluation of an anti-GD2 MoAb, 14G2a, has been initiated in malignant melanoma, neuroblastoma, and small cell carcinoma of the lung. Based on reports that the MoAb 17-1A has activity in pancreatic cancer, the ECOG is about to initiate a Phase II study to define response rate and duration.

11) Ifosfamide

Became commercially available for refractory testicular carcinoma.

12) SR 2508

A randomized trial in patients with head and neck cancer is ongoing to establish the efficacy of SR 2508 and radiotherapy versus radiotherapy alone. Several pilot studies are ongoing. A Phase I trial is seeking to define the maximally tolerated dose of SR 2508 when given with brachytherapy based on preclinical data which suggest that SR 2508 may be more effective when given with low dose rate radiotherapy. Patient accrual continues in a Phase II trial in prostate cancer which seeks to establish whether patients treated with SR 2508 and radiotherapy have a higher local control rate than one might anticipate.

Two Phase I trials are going in patients with refractory solid tumors to define the MTD of SR 2508 when given with cyclophosphamide. In addition, a Phase I trial in patients with CLL will seek to determine the MTD in this patient population. Pharmacokinetic and pharmacodynamic studies are part of these proposed trials and seek to establish the mechanism of the proposed chemosensitization.

13) Amonafide

Broad Phase II evaluation of this agent is underway. Although the data concerning activity are not yet available, early toxicity data suggest that this drug is well tolerated with reversible myelosuppression as its dose limiting toxicity. Objective responses have been observed in a Phase II trial in patients with breast cancer; a second Phase II trial is ongoing to define the level of activity of this compound in this disease site more precisely.

14) WR 2721

Data from the University of Pennsylvania suggest that WR 2721 and cisplatin is an active regimen in the treatment of melanoma. A 53% objective response rate was observed in 36 patients with metastatic melanoma treated with this two drug combination, including 5 objective responses among 6 patients treated with WR 2721 and cisplatin 150 mg/m<sup>2</sup>. Based on these promising data, a randomized Phase III trial is ongoing in the ECOG. Because WR 2721 may increase the cytotoxicity of cisplatin, as well as ameliorate its toxicity, Phase II trials of this two drug combination are anticipated in patients with cancers of the breast and prostate.



15) Taxol

This unique natural product derived from the bark of Taxus brevifolia has shown promising antitumor activity. A 33% response rate has been observed in patients with refractory ovarian cancer. A Phase II trial has been undertaken by the GOG to confirm these preliminary data. A Phase I trial of cisplatin and Taxol is ongoing which will define the dose limiting toxicities of this two drug combination; if warranted, a Phase III comparison of cisplatin versus cisplatin and Taxol will be undertaken in newly diagnosed patients.

Broad Phase II screening will be undertaken when additional drug supply becomes available.

16) L-Buthionine Sulfoximine

The drug inhibits glutathione biosynthesis and causes a depletion of cellular glutathione levels. The drug has been shown to reverse the induced resistance of human ovarian cell lines to melphalan. The initial clinical studies will be Phase I studies of the combination of BSO and Melphalan.

17) Fostriecin

This novel compound, produced by Streptomyces pulvaraeus, inhibits macromolecular synthesis and is thought to inhibit DNA topoisomerase II. Additional preclinical data suggest that fostriecin enters cells by the reduced folate carrier system. Because of its unique structure, novel proposed mechanism of action, and need for the reduced folate carrier system to gain cell entry, fostriecin was chosen for further evaluation. Clinical trials are anticipated to begin in the near future.

18) Porfiromycin

This is an N-methyl derivative of mitomycin C. Both porfiromycin and mitomycin underwent clinical evaluation in the 1960's. Since both compounds demonstrated a similar spectrum of clinical antitumor activity and mitomycin C is more potent than porfiromycin, the clinical development of porfiromycin was not pursued, and the DCT closed the IND for porfiromycin in the early 1970's. Preclinical data by Sartorelli et al. suggest that porfiromycin is preferentially toxic to hypoxic cells compared to well oxygenated cells. Based on these data, investigators at Yale University will shortly initiate a clinical trial of porfiromycin in patients with head and neck carcinoma who are undergoing radiotherapy. The Decision Network voted to reopen the IND for porfiromycin in May, 1988 for limited clinical evaluation for this indication.

19) Trimetrexate (TMTX)

A randomized clinical trial to test the value of murine data in predicting schedule dependency was performed. Based on murine data demonstrating superior antileukemic activity when TMTX was administered on an every 3 hr x 8 doses days 1, 5, 9 schedule, the daily x 5 schedule was chosen for the broad Phase II screening of TMTX. A direct clinical comparison of the dx5 schedule versus the every other week schedule in colon cancer produced responses only on the every other week arm. Broad Phase II screening of TMTX has revealed activity on a dx5 schedule (4 PR in 24 patients) in soft tissue sarcoma patients who had failed a prior adriamycin-containing regimen). Phase II investigation of the every other week schedule in sarcoma is now being pursued. A Phase I pharmacokinetic trial in patients with hepatic/renal dysfunction is planned, and a Phase I trial of TMTX/cisplatin is underway which will evaluate the effect of cisplatin on the renal excretion of TMTX and its active metabolites. TMTX continues to produce excellent responses against *Pneumocystis carinii* pneumonia in trials performed by Masur and Allegra.

20) Flavone Acetic Acid

The antitumor activity of FAA in animal models is primarily related to induction of interferon and other cytokines, resulting in enhanced natural killer activity. However a Phase I trial conducted at the BRMP failed to demonstrate any immune modulation in humans despite reaching MTD doses. Chronic administration trials have been conducted in Europe, but these Phase I trials have not reported antitumor effects. If achievement of higher peak in vivo levels (by decreasing the duration of administration to 1 or 3 hours), and deleting alkalization (which abrogates activity in animal models) does not reproduce the clinical and immunologic effects seen in the mice, clinical development of this agent will cease.

21) Teniposide

VM-26 has become an important component of therapy for acute lymphoblastic leukemia/lymphoma and for neuroblastoma. A Group C protocol has been approved by the FDA for VM-26 in combination with Ara-C for the treatment of patients with relapsed or refractory acute lymphoblastic leukemia. Two confirmatory trials in small cell lung cancer have been initiated based on data from the Finsen Institute which demonstrated extraordinary single agent activity of VM-26 (J Clin Oncol 4:524, 1986).

22) Liposomal Doxorubicin

Phase I trials with liposomal doxorubicin supplied by The Liposome Co. are being initiated by 3 contractors on weekly or every 3 week schedules. Preclinical studies with liposome-encapsulated doxorubicin have shown that the maximally tolerated dose of doxorubicin can be increased by approximately 2.5 fold. This has been accompanied by an alteration in the tissue distribution of doxorubicin, with less accumulation in cardiac tissue. Superior antitumor activity has been noted in some, but not all, preclinical models.

23) Fazarabine

Phase I trials of this analog of both Ara-C and 5-azacytidine have been completed and Phase II trials are beginning.

24) PALA/5-FU

The investigational agent, PALA, is being tested in Phase II trials at low dose ( $250 \text{ mg/m}^2$ ) with FUra ( $2600 \text{ mg/m}^2/24 \text{ hr}$  following PALA) in colon, gastric and pancreatic cancers to confirm the impressive results seen in a previous Phase II trial (2 CR, 14 PR in 37 evaluable patients). This regimen will also be included in randomized Phase III trials to determine the contribution of PALA.

25) PROSORBA column

A recent report claimed therapeutic responses with three times weekly pheresis using a STAPH protein A column. The most consistent responses were noted in breast cancer. The NCI will sponsor a phase 2 study of this treatment modality in patients with advanced breast cancer. Immunologic monitoring will be an integral part of this study to determine the mechanism of anti-tumor responses. Rational combinations with other biologics will then be pursued.

26) Deoxycoformycin (dCF)

Phase III trials of dCF versus alpha-interferon are accruing well. In Phase II trials, dCF has produced remissions (CR + PR) in 85% of hairy cell leukemia patients (99 CR, 46 PR of 170 Phase II patients). Application Group C designation of pentostatin for Hairy Cell Leukemia patients refractory to alpha-interferon has been approved by the FDA. The drug is currently available outside clinical trials through Group C protocols. In Phase II trials, dCF is a 26% agent (3CR, 48 PR of 196 patients) in CLL. Combination trials with FAMP and chlorambucil are planned in CLL.

A Phase I trial in patients with impaired renal function has been initiated. A combination trial of dCF and alpha-interferon in mycosis fungoides and a trial of single agent dCF for the treatment of acute graft versus host disease after bone marrow transplant have also been initiated. An ex vivo purging trial to take advantage of the selective cytotoxicity of dCF with deoxyadenosine for T-lymphocytes is planned.

27) Piroxantrone (formerly Oxantrazole)

Piroxantrone is one of a new class of anthrapyrazole intercalating agents which is being co-developed with Warner-Lambert just finished Phase I testing. This was the first NCI agent to utilize Phase I dose escalations based on pharmacokinetics according to the Blood Level Working Group method and it is estimated that 9-12 fewer patients were required for the Phase I. Piroxantrone will now undergo broad Phase II testing at a dose of 160 mg/m<sup>2</sup> iv bolus every three weeks. Initial Phase II trials should begin accruing patients shortly.

28) Deoxysperqualin

Deoxysperqualin remains in Phase I. A DLT of hypotension was identified at doses of 2100-2792 mg/m<sup>2</sup>/dx5 continuous infusion. These dose levels produced plasma concentrations which approached those which were active in vitro. While myelosuppression and gastrointestinal mucosal toxicity have also been seen, these have not been clearly dose related. Current plans include Phase II testing in diseases where responses were seen in Phase I (primarily squamous carcinomas of head and neck and lung, and possibly cervix) or where there was a suggestion of in vitro activity (breast cancer). In addition, a 7 day infusion will be explored in an attempt to increase patient exposure (AUC) and, perhaps, arrive at a more marrow toxic dose that might be employed in leukemia studies.

29) Ipomeanol

Three Phase I trials are currently active: single bolus q 21 days at Johns Hopkins and daily x 5 bolus q 21 days at NCI-Navy and Ohio State. The latter trials have only recently begun accrual. 4 dose escalations have been completed on the single bolus trial; results are as follows:

1. Twelve patients have been treated. There has been no clinically apparent pulmonary toxicity, however, reversible and modest increases in lung density (as assessed by CT scan) and/or decrements in DLCO/FEV<sub>1</sub> have occurred in 10 courses administered to 7 patients.

- a. 5 courses were associated with increased lung density and 5 had both increased density and decreased DLCO/FEV<sub>1</sub>.
- b. 2 infusions in 1 patient were associated with mild hypertension.
- c. While the dose in mg/m<sup>2</sup> represents approximately 70% of the LD<sub>10</sub> in female mice, the AUCs at that dose represent only 4% of the AUC at the LD<sub>10</sub> in female mice and 10% of the AUC at the lowest non-toxic dose in dogs which suggests that human handling of the compound may be somewhat different. Discussions regarding alternate (pharmacologically based) dose escalations are underway.

After some initial experience is gained with the daily x 5 schedule, trials designed to induce tolerance will be initiated.

### 30) 10-Edam

Data suggests that, while a DHFR inhibitor like MTX, EDAM has more efficient intracellular transport; concentrates preferentially in tumor tissue; undergoes more extensive polyglutamylation; is more active than MTX against a number of murine tumors and human tumor xenografts; is clinically active in some tumors where MTX is inactive such as NSCLC and shows preclinical in vivo synergy with alkylators.

Negotiations with Ciba-Geigy regarding the collaborative development of 10-EDAM have been ongoing for the past 18 months. Because this will be the first collaboration between Ciba-Geigy and the NCI, much time has been spent negotiating a secrecy agreement and a working agreement for NCI sponsorship of trials. Ciba officials gave corporate approval to the collaboration several months ago and expressed enthusiasm about the relationship, however, the legal details remain under discussion.

Once a satisfactory agreement is reached, NCI will cross-file with the Ciba IND, we anticipate that this will occur within the next three months) and discuss a complementary development plan which avoids duplication. It is anticipated that, in addition to completing a broad Phase II screen, high dose trials with leucovorin rescue and combination trials (designed to evaluate the preclinical synergy with alkylators) will be explored.

### 31) Carboplatin

Bristol-Myers filed a successful NDA for this compound in the past year. CBDCA is now commercially available with an indication for the second-line treatment of ovarian carcinoma. The results of a front-line trial were presented at ASCO and failed to demonstrate that cisplatin is superior to CBDCA in the first-line therapy of ovarian cancer. The results of the other pivotal NDA-directed first-line ovarian study in the National Cancer Institute of Canada have not yet been published, however, it is likely that the FDA will review the first-line indication again in the near future in light of the SWOG data.

### 32) Suramin

Suramin is a polysulfonated naphthylurea which has been in clinical use since 1920 for the treatment of trypanosomiasis. Interest in its antitumor effects were stimulated by the finding that suramin caused Addison's Disease in AIDS patients and by subsequent work which demonstrated that it was a growth factor antagonist. The activity of a number of growth factors appears to be blocked by suramin, including basic-FGF, PDGF, TGF-beta and EGF.

Toxicity includes thrombocytopenia (in previously treated patients), coagulopathy, mild alterations in renal function and neurotoxicity (polyradiculopathy progressing to flaccid paralysis requiring intensive care unit support at high serum levels). These toxicities, which could prevent use of the agent, appear to be quite manageable with dose adjustments made on the basis of pharmacologic monitoring to maintain serum suramin levels <300 mcg/ml and careful monitoring of the prothrombin time to maintain it at  $\leq 17.5$  seconds. Other toxicities have included proteinuria, vortex keratopathy, rash, anorexia/malaise, hepatitis and adrenal insufficiency.

Responses to suramin have been seen in adrenal and renal cancers and, most recently, in a significant proportion of the prostate cancer patients treated on the intramural broad Phase II protocol. As a result of the dissemination of these early trial results, interest in this compound has grown both within the extramural investigator community and on the part of the drug company. The CTEP has received numerous Letters of Intent (primarily in prostate cancer) and many additional inquiries within the past few months.

Blood level monitoring will be done weekly on all patients on Phase II trials so that infusion doses can be individualized to maintain levels >200 and <300 mcg/ml and, therefore, ensure that therapeutic levels are achieved as rapidly as possible while reducing the risk of neurotoxicity which correlated with peak

levels >300mcg/ml. Serum suramin levels will be measured by the method of Klecker and Collins. The method requires organic extraction of the sample followed by reverse phase HPLC with tetrabutylammonium phosphate as an ion-pairing reagent.

Immediate Plans Include the Following:

1. Rapid confirmation of the clinical activity and toxicity profile demonstrated in the intramural program through Inter-contract studies in measurable prostate cancer and adrenal cancer and a randomized study of suramin versus low-dose steroid therapy in non-measurable prostate cancer. In addition, a multi-institutional study in prostate cancer, which incorporates correlative tumor biology (growth factor inhibition, etc.) will be initiated. All trials will include weekly blood level monitoring to individualize patient dose.
2. Additional Phase I work will be done to attempt to define a schedule which produces the desired blood levels with reduced toxicity and greater patient convenience.

33) HMBA

Three Phase II trials of this polar-planar differentiating agent in myelodysplastic syndrome and one in malignant melanoma are currently ongoing. The implementation of biologic endpoints cytogenetics, measurement of early- or late- myeloid antigen levels, multilineage bone marrow progenitor cell assays, expression of proto-oncogenes (c-myc, c-fos, c-fms are an intrinsic part of each of these trials. The Phase I oral study (between Walter Reed, CTEP and the intramural program) with comparative pharmacology studies between parenteral formulation and oral formulations (either solution or tablets) has been completed.

34) Fludarabine Phosphate (FAMP)

The drug has demonstrated significant single agent activity in alkylator-refractory lymphoproliferative disorders, especially in chronic lymphocytic leukemia (CLL) and favorable histology non-Hodgkins lymphoma. Pilot trials of FAMP-containing combination regimens (FAMP + Prednisone, FAMP + chlorambucil, and FAMP + deoxycoformycin) in CLL are being activated. Application for Group C designation of the drug (for refractory CLL) is underway.

35) Merbarone

Phase I trials of Merbarone using either 5-day continuous infusion or 2-hour infusion daily for 5 days are being completed. A number of Phase II trials in a panel of solid tumors are now being planned.

36) Dihydroleupenone

This is the first compound chosen for clinical trials based on its activity in the human tumor colony-forming assay; the highest response rate (27%) in this assay was seen in lung cancers. A Phase I trial in lung cancer patients (disease-oriented) is nearly completed at the Navy.

37) Chloroquinoxaline Sulfonamide

This is the second compound with outstanding activity in the human tumor cloning assay. The compound is especially active preclinically against melanoma, ovary, breast, and lung tumors. Phase I trials have been started using 1-hour infusion schedule. Based on drug's pharmacokinetic characteristics with the initial schedule of administration, other schedules would be explored.

B. Distribution of Investigational Agents

1) Drug Accountability

The drug accountability system, implemented in January 1983, has continued to function well. All investigational drugs must be ordered and dispensed to patients by protocol and be documented on an investigational drug accountability form. This has proven to be an essential addition to the site visit monitoring as conducted by the Quality Assurance and Compliance Section. This form has been accepted by several drug companies for use in many of the cancer centers across the country. During the past year we have added a drug transfer form to the NCI Investigational Drug Accountability procedure and have received OMB renewal for the Drug Accountability and drug transfer forms. We have worked closely with the NCI Information Resources Branch to reprint the revised Drug Accountability procedures book. The Drug Accountability form and the drug transfer form have helped to reduce the amount of unused drug which must be returned to NCI.



2) Electronic Clinical Drug Request

The section has developed an electronic clinical drug request system for the transmission of drug requests from investigators to NCI. After the system was designed, equipment was purchased and a User's Guide was written. A pilot project was initiated at two major cancer centers: Memorial-Sloan Kettering and M.D. Anderson. The pilot was later expanded to more diverse clinical practice settings, nationwide, bridging several different time zones. These pilot programs have been successful in simplifying the drug ordering procedure and reduced overall drug distribution time from weeks to days. We have begun to offer the Electronic Clinical Drug Request system to investigators, nationwide. The procedure has been well received and has helped to minimize the need for investigators to maintain large drug inventories and it has thus helped reduce drug cost to NCI.

3) Computer Costs and Planned System Changes

The DMAS continues to be concerned about the high Computer Costs associated with the current computer system used to monitor the distribution of drugs. In the past year we conducted an analysis of this system. As a result we have received Concept Approval to upgrade it to a state-of-the-art system using a file server, networked PC's and significantly reduce the use of DCRT. Significant enhancements will be added to the system in the coming years which will have of net effect of reduced costs as well as improving system efficiencies and responsiveness to DMAS and CTEP.

4) Investigator Registration

The administration of the annual reregistration of investigators continues to be an important function of DMAS. The investigator compliance is presently 100%. Also, during the past year we have completed the implementation the new FDA-1572 forms. Because of the changed format and content of the form and the requirement of more detail, greater interaction with investigators has been required to explain the form changes.

5) Drug Cost Expenditures

The total drug costs have decreased from \$ 3.5 million in FY'86 to \$2.9 million in FY'87. Costs have increased as expected in FY'88 to \$ 4.0 million as a result of increased drug distribution to Cooperative Groups, CCOP's, the NCI intramural program and NIAID for AIDS. These increasing trends are continuing in FY 89 and are expected to total \$4.5 million.

#### 6) Drug Distribution Data for the Past Year

Number of Drug Orders ( <u>Line Items</u> )	Number New Special Exception Protocols ( <u>Reorders</u> )	New Group C <u>Orders</u>	Total Containers (Vials/ampules bottles) <u>Distributed</u>
15,504 (29,904)	696 (778)	550	1,252,986

### BIOLOGICS EVALUATION SECTION

#### Alpha Interferon

Although licensed in this country for Hairy Cell Leukemia and Kaposi's Sarcoma, investigational studies in a variety of disease types are still being pursued. Adjuvant studies in renal cell carcinoma, melanoma and ovarian cancer are still ongoing.

Preclinical studies have indicated that IL-2 and alpha IFN have synergistic antitumor activity when administered together and a recently completed Phase I trial at the NCI documented antitumor activity in patients with renal cell carcinoma and malignant melanoma. Recently, a high response rate was observed in previously untreated patients with advanced colorectal carcinoma who received 5-FU in combination with alpha IFN. The results of these studies are currently being confirmed.

Recently interferon-alpha in combination with 5-fluorouracil showed promising activity in patients with untreated metastatic colorectal cancer. Confirmatory studies have been initiated, and the activity of this combination in other GI malignancies are being performed under the CTEP phase II/III contract.

#### Gamma Interferon

Previous clinical trials conducted by the NCI identified a dose and schedule for gamma interferon administration which resulted in optimal biological activity. Large scale adjuvant trials which use this regimen of gamma interferon administration are currently in progress in malignant melanoma, colon and small cell carcinoma of the lung. Two of these trials are expected to complete accrual by the end of this year, although follow-up for response and survival will continue. Monocyte function of patients treated on these studies is also being measured and will be correlated with antitumor activity. A recent Phase II study has revealed that 3 SCLC patients who were partial responders to PACE chemotherapy converted to CR status following IFN-gamma administration. Two Phase II trials of IFN-alpha + IFN-gamma in CML are ongoing as is a trial of ex vivo treatment of bone marrow with IFN-gamma during bone marrow transplantation of CML patients.

## IL-2

IL-2 alone, using a high dose bolus schedule, produced complete and partial responses in 10/47 (21%) melanoma patients. Phase II trials in a number of disease sites (renal, colon, lymphoma, breast, pancreas, lung) have opened using a less toxic continuous infusion schedule of IL-2. However accrual to these studies is threatened by inability of investigators to receive reimbursement from insurance companies for prolonged hospital stays. Pediatric Phase II trials using a similar schedule are set to open.

## IL-2/LAK

1. Patients with melanoma or renal cell cancer who have obtained complete remissions, following IL-2/LAK remain in unmaintained remission at 12-36 + months following treatment. Approximately 5% of patients achieve complete remissions with treatment.
2. A randomized study in renal cell cancer patients comparing continuous infusion versus bolus administration of IL-2 in combination with LAK cells found no significant difference in response or toxicity. Response rates were approximately 20% in both arms. However successive Phase II studies demonstrated that continuous infusion IL-2 with LAK cells was ineffective in melanoma patients, while bolus administration of IL-2 with or without LAK produced response rates of 20-25%.

The Modified Group C program has been safely initiated at 14 NCI Clinical and Comprehensive Cancer Centers and has been accruing 10 patients per month. The Modified Group C randomized study of IL-2 versus IL-2/LAK, to determine if LAK contributes to the efficacy of IL-2, continues to accrue patients. Rosenberg et al will publish the results of a similar randomized trial, which showed no statistical differences between arms but suggested an increased complete response rate when LAK were administered with IL-2. Ongoing BRMP trials are studying means of generating effector LAK cells of greater potency.

## IL-2/TIL

The Surgery Branch published their results with IL-2/TIL therapy. Although the reported response rates are quite high in melanoma (11/20), this therapy is limited by the ability to obtain tumor from patients and expand the infiltrating lymphocytes into a large number of highly specific tumor effector cells. The NCI is sponsoring TIL trials with extensive basic science corollary laboratory studies to further define the properties of these cells. Other methods of generating antigen specific cells are being pursued, including the use of in vivo active specific immunotherapy (vaccination with autologous tumor) followed by further ex vivo expansion of these antigen primed cells with autologous tumor. In animal models this method has resulted in generation of antigen specific cells even from non-immunogenic tumors.

In the past year several trials of active specific immunotherapy in combination with IL-2 have been approved. In melanoma both autologous and allogeneic tumor cell vaccines are being explored in combination with IL-2 in patients with advanced disease. A similar study in metastatic renal cell cancer using an autologous tumor vaccine has begun.

Trials of IL-2 in combination with chemotherapy are proceeding. High dose IL-2 followed in sequence by high dose cis-platinum produced a 40% response rate, but with significant toxicity. Other schedules combining cisplatin and IL-2 are under investigation. Two trials of adriamycin with IL-2 are ongoing. Based on marked preclinical synergy and initial reports of clinical anti-tumor activity the NCI has sponsored combination trials with cyclophosphamide and IL-2. The results on these Phase II studies are pending. Several trials of combination chemotherapy with IL-2 in melanoma have been initiated in hopes that combining two active modalities will lead to overall improved response rates and response durations.

Other innovative trials involve the use of IL-2 as a marrow and immune cell protectant when administered with high dose chemotherapy. Based on elegant pre-clinical models the first study of high dose chemotherapy, followed by autologous marrow incubated in IL-2 and returned to the patient, was approved. In the animal models effector cells could be generated in the marrow which mediated antitumor effects when infused into the animal and combined with systemic IL-2 administration.

Adjuvant studies have also been considered and approved. A cooperative group plans to administer systemic IL-2 to patients with acute myelogenous leukemia in second complete remission. The toxicity of high dose regimens has hindered attempts to perform adjuvant trials in diseases where IL-2 has been active in the advanced setting.

Trials employing alternate routes of administration are in progress including intratumor, intraperitoneal, hepatic and splenic intraarterial, and intracavitary brain.

### Tumor Necrosis Factor

Broad Phase II testing continues to define the antitumor activity of TNF when given as a single agent. Other specific areas of interest that are being pursued include: combination studies with topoisomerase II inhibitors or IL-2 based on preclinical evidence of synergistic antitumor activity; pediatric Phase I trials of TNF alone or with a topoisomerase II agent; and Phase I trial of intravesical TNF for bladder cancer.

### Combination Cytokine Regimens

IL-2 in combination with interferon-alfa appears to be a promising regimen. Initial trials indicate increased response rates in melanoma and perhaps renal cell cancer. In renal cell cancer lower doses of these agents, which can be administered in the outpatient setting, may be active. Phase II trials are in progress, and high dose comparative trials in renal cell carcinoma and melanoma (IL-2 versus IL-2/IFN) are set to begin in the IL-2/LAK contract centers.

Phase I trials of TNF followed in sequence by IL-2 are ongoing. A Phase I trial of concurrent administration of these agents was completed, and antitumor activity was noted in non-small-cell lung cancer. Phase II trials in a limited number of disease sites are being solicited.

Phase I trials of IL-2 in combination with interferon gamma are near completion. These trials will be reviewed and the most active schedules (immune enhancement and antitumor activity) will be explored further in Phase II trials.

### Colony Stimulating Factors

Early studies have also shown that G-CSF and GM-CSF can reduce the neutropenia associated with chemotherapy, bone marrow transplantation, and some congenital or acquired hematopoietic deficiency states. To reduce toxicity or to permit administration of more treatment, the NCI is adding CSFs to active chemotherapy regimens used for the treatment of breast cancer, lung cancer, ovarian cancer, multiple myeloma, lymphoma, testicular carcinoma, and sarcomas. CSFs are also being tested in combination with bone marrow transplantation to shorten the time to hematopoietic reconstitution as well as to augment harvest of progenitor cells from the peripheral blood. In addition, CSF's are being used as growth factors in patients with relapsed/refractory leukemia in an attempt to recruit quiescent malignant cells in cell cycle to increase responses to standard cell-cycle specific therapy.

### Levamisole

The results of a recent trial from the NCCTG using 5-FU + levamisole as adjuvant therapy for resected colon cancer suggested that this combination may prolong the disease-free and overall survival of patients with Dukes' C disease. This observation is being studied in a confirmatory Intergroup trial; the NCI and FDA have designated 5-FU/levamisole as a Group C/Treatment IND.

### Monoclonal Antibodies (MoABs)

A protocol to be used at NCI contract institutions has been developed to permit rapid comparison of multiple MoABs directed against the same antigen. This approach will be used to determine the optimal variable region for future MoAB constructs and will initially study MoABs directed against the CEA and TAG-72 antigen in patients with colorectal carcinoma. An anti-CD3 MoAB will be assessed

for enhancement of T-cell function in patients with solid malignancies. Protocols have been initiated to evaluate MoAb-toxin conjugates. An anti-CD5 MoAb conjugated to ricin A chain, previously used to deplete T-cell populations in patients with CD5 (+) neoplasms. In addition, an anti-ovarian carcinoma MoAb conjugated to pseudomonas exotoxin is undergoing clinical evaluation. Studies to further define the activity of the anti-GD3 MoAb, R24 in malignant melanoma are currently in progress. The clinical evaluation of an anti-GD2 MoAb, 14G2a, has been initiated in malignant melanoma, neuroblastoma, and small cell carcinoma of the lung. Based on reports that the MoAb 17-1A has activity in pancreatic cancer, the ECOG is about to initiate a Phase II study to define response rate and duration.

## DEVELOPMENTAL CHEMOTHERAPY SECTION

### Ifosfamide

Became commercially available for refractory testicular carcinoma.

### SR 2508

A randomized trial in patients with head and neck cancer is ongoing to establish the efficacy of SR 2508 and radiotherapy versus radiotherapy alone. Several pilot studies are ongoing. A Phase I trial is seeking to define the maximally tolerated dose of SR 2508 when given with brachytherapy based on preclinical data which suggest that SR 2508 may be more effective when given with low dose rate radiotherapy. Patient accrual continues in a Phase II trial in prostate cancer which seeks to establish whether patients treated with SR 2508 and radiotherapy have a higher local control rate than one might anticipate.

Two Phase I trials are going in patients with refractory solid tumors to define the MTD of SR 2508 when given with cyclophosphamide. In addition, a Phase I trial in patients with CLL will seek to determine the MTD in this patient population. Pharmacokinetic and pharmacodynamic studies are part of these proposed trials and seek to establish the mechanism of the proposed chemosensitization.

### Amonafide

Broad Phase II evaluation of this agent is underway. Although the data concerning activity are not yet available, early toxicity data suggest that this drug is well tolerated with reversible myelosuppression as its dose limiting toxicity. Objective responses have been observed in a Phase II trial in patients with breast cancer; a second Phase II trial is ongoing to define the level of activity of this compound in this disease site more precisely.

### WR2721

Data from the University of Pennsylvania suggest that WR2721 and cisplatin is an active regimen in the treatment of melanoma. A 53% objective response rate was observed in 36 patients with metastatic melanoma treated with this two drug combination, including 5 objective responses among 6 patients treated with WR2721 and cisplatin 150 mg/m<sup>2</sup>. Based on these promising data, a randomized Phase III trial is ongoing in the ECOG. Because WR2721 may increase the cytotoxicity of cisplatin, as well as ameliorate its toxicity, Phase II trials of this two drug combination are anticipated in patients with cancers of the breast and prostate.

### Taxol

This unique natural product derived from the bark of Taxus brevifolia has shown promising antitumor activity. A 33% response rate has been observed in patients with refractory ovarian cancer. A Phase II trial has been undertaken by the GOG to confirm these preliminary data. A Phase I trial of cisplatin and Taxol is ongoing which will define the dose limiting toxicities of this two drug combination; if warranted, a Phase III comparison of cisplatin versus cisplatin and Taxol will be undertaken in newly diagnosed patients. Broad Phase II screening will be undertaken when additional drug supply becomes available.

### L-Buthionine Sulfoximine

The drug inhibits glutathione biosynthesis and causes a depletion of cellular glutathione levels. The drug has been shown to reverse the induced resistance of human ovarian cell lines to melphalan. The initial clinical studies will be Phase I studies of the combination of BSO and Melphalan.

### Fostriecin

This novel compound, produced by Streptomyces pulvaraeus, inhibits macromolecular synthesis and is thought to inhibit DNA topoisomerase II. Additional preclinical data suggest that fostriecin enters cells by the reduced folate carrier system. Because of its unique structure, novel proposed mechanism of action, and need for the reduced folate carrier system to gain cell entry, fostriecin was chosen for further evaluation. Clinical trials are anticipated to begin in the near future.

### Porfiromycin

This is an N-methyl derivative of mitomycin-C. Both porfiromycin and mitomycin underwent clinical evaluation in the 1960's. Since both compounds demonstrated a similar spectrum of clinical antitumor activity and mitomycin C is more potent than porfiromycin, the clinical development of porfiromycin was not pursued, and the DCT closed the IND for porfiromycin in the early 1970's. Preclinical data by

Sartorelli et al. suggest that porfiromycin is preferentially toxic to hypoxic cells compared to well oxygenated cells. Based on these data, investigators at Yale University will shortly initiate a clinical trial of porfiromycin in patients with head and neck carcinoma who are undergoing radiotherapy. The Decision Network voted to reopen the IND for porfiromycin in May, 1988 for limited clinical evaluation for this indication.

### Trimetrexate (TMTX)

A randomized clinical trial to test the value of murine data in predicting schedule dependency was performed. Based on murine data demonstrating superior antileukemic activity when TMTX was administered on an every 3 hr x 8 doses days 1, 5, 9 schedule, the daily x 5 schedule was chosen for the broad Phase II screening of TMTX. A direct clinical comparison of the dx5 schedule versus the every other week schedule in colon cancer produced responses only on the every other week arm. Broad Phase II screening of TMTX has revealed activity on a dx5 schedule (4 PR in 24 patients) in soft tissue sarcoma patients who had failed a prior adriamycin-containing regimen). Phase II investigation of the every other week schedule in sarcoma is now being pursued. A Phase I pharmacokinetic trial in patients with hepatic/renal dysfunction is planned, and a Phase I trial of TMTX/cisplatin is underway which will evaluate the effect of cisplatin on the renal excretion of TMTX and its active metabolites. TMTX continues to produce excellent responses against *Pneumocystis carinii* pneumonia in trials performed by Masur and Allegra.

### Flavone Acetic Acid

The antitumor activity of FAA in animal models is primarily related to induction of interferon and other cytokines, resulting in enhanced natural killer activity. However a Phase I trial conducted at the BRMP failed to demonstrate any immune modulation in humans despite reaching MTD doses. Chronic administration trials have been conducted in Europe, but these Phase I trials have not reported antitumor effects. If achievement of higher peak in vivo levels (by decreasing the duration of administration to 1 or 3 hours), and deleting alkalization (which abrogates activity in animal models) does not reproduce the clinical and immunologic effects seen in the mice, clinical development of this agent will cease.

### Teniposide

VM-26 has become an important component of therapy for acute lymphoblastic leukemia/lymphoma and for neuroblastoma. A Group C protocol has been approved by the FDA for VM-26 in combination with Ara-C for the treatment of patients with relapsed or refractory acute lymphoblastic leukemia. Two confirmatory trials in small cell lung cancer have been initiated based on data from the Finsen Institute which demonstrated extraordinary single agent activity of VM-26 (J Clin Oncol 4:524, 1986).



### Liposomal Doxorubicin

Phase I trials with liposomal doxorubicin supplied by The Liposome Co. are being initiated by 3 contractors on weekly or every 3 week schedules. Preclinical studies with liposome-encapsulated doxorubicin have shown that the maximally tolerated dose of doxorubicin can be increased by approximately 2.5 fold. This has been accompanied by an alteration in the tissue distribution of doxorubicin, with less accumulation in cardiac tissue. Superior antitumor activity has been noted in some, but not all, preclinical models.

### Fazarabine

Phase I trials of this analog of both Ara-C and 5-azacytidine have been completed and Phase II trials are beginning.

### PALA/5-FU

The investigational agent, PALA, is being tested in Phase II trials at low dose (250 mg/m<sup>2</sup>) with FUra (2600 mg/m<sup>2</sup>/24 hr following PALA) in colon, gastric and pancreatic cancers to confirm the impressive results seen in a previous Phase II trial (2 CR, 14 PR in 37 evaluable patients). This regimen will also be included in randomized Phase III trials to determine the contribution of PALA.

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After some initial experience is gained with the daily x 5 schedule, trials designed to induce tolerance will be initiated.

## 10-Edam

Data suggests that, while a DHFR inhibitor like MTX, EDAM has more efficient intracellular transport; concentrates preferentially in tumor tissue; undergoes more extensive polyglutamylation; is more active than MTX against a number of murine tumors and human tumor xenografts; is clinically active in some tumors where MTX is inactive such as NSCLC and shows preclinical in vivo synergy with alkylators.

Negotiations with Ciba-Geigy regarding the collaborative development of 10-EDAM have been ongoing for the past 18 months. Because this will be the first collaboration between Ciba-Geigy and the NCI, much time has been spent negotiating a secrecy agreement and a working agreement for NCI sponsorship of trials. Ciba officials gave corporate approval to the collaboration several months ago and expressed enthusiasm about the relationship, however, the legal details remain under discussion.

Once a satisfactory agreement is reached, NCI will cross-file with the Ciba IND, we anticipate that this will occur within the next three months) and discuss a complementary development plan which avoids duplication. It is anticipated that, in addition to completing a broad Phase II screen, high dose trials with leukovorin rescue and combination trials (designed to evaluate the preclinical synergy with alkylators) will be explored.

## Carboplatin

Bristol-Myers filed a successful NDA for this compound in the past year. CBDCA is now commercially available with an indication for the second-line treatment of ovarian carcinoma. The results of a front-line trial were presented at ASCO and failed to demonstrate that cisplatin is superior to CBDCA in the first-line therapy of ovarian cancer. The results of the other pivotal NDA-directed first-line ovarian study in the National Cancer Institute of Canada have not yet been published, however, it is likely that the FDA will review the first-line indication again in the near future in light of the SWOG data.

## Suramin

Suramin is a polysulfonated naphthylurea which has been in clinical use since 1920 for the treatment of trypanosomiasis. Interest in its antitumor effects were stimulated by the finding that suramin caused Addison's Disease in AIDS patients and by subsequent work which demonstrated that it was a growth factor antagonist. The activity of a number of growth factors appears to be blocked by suramin, including basic-FGF, PDGF, TGF-beta and EGF.

Toxicity includes thrombocytopenia (in previously treated patients), coagulopathy, mild alterations in renal function and neurotoxicity (polyradiculopathy progressing to flaccid paralysis requiring intensive care unit support at high serum levels). These toxicities, which could prevent use of the agent, appear to be quite manageable with dose adjustments made on the basis of pharmacologic monitoring to maintain serum suramin levels <300 mcg/ml and careful monitoring of the prothrombin time to maintain it at  $\leq 17.5$  seconds. Other

toxicities have included proteinuria, vortex keratopathy, rash, anorexia/malaise, hepatitis and adrenal insufficiency.

Responses to suramin have been seen in adrenal and renal cancers and, most recently, in a significant proportion of the prostate cancer patients treated on the intramural broad Phase II protocol. As a result of the dissemination of these early trial results, interest in this compound has grown both within the extramural investigator community and on the part of the drug company. The CTEP has received numerous Letters of Intent (primarily in prostate cancer) and many additional inquiries within the past few months.

Blood level monitoring will be done weekly on all patients on Phase II trials so that infusion doses can be individualized to maintain levels  $>200$  and  $<300$  mcg/ml and, therefore, ensure that therapeutic levels are achieved as rapidly as possible while reducing the risk of neurotoxicity which correlated with peak levels  $>300$ mcg/ml. Serum suramin levels will be measured by the method of Klecker and Collins. The method requires organic extraction of the sample followed by reverse phase HPLC with tetrabutylammonium phosphate as an ion-pairing reagent.

Immediate Plans Include the Following:

1. Rapid confirmation of the clinical activity and toxicity profile demonstrated in the intramural program through Inter-contract studies in measurable prostate cancer and adrenal cancer and a randomized study of suramin versus low-dose steroid therapy in non-measurable prostate cancer. In addition, a multi-institutional study in prostate cancer, which incorporates correlative tumor biology (growth factor inhibition, etc.) will be initiated. All trials will include weekly blood level monitoring to individualize patient dose.
2. Additional Phase I work will be done to attempt to define a schedule which produces the desired blood levels with reduced toxicity and greater patient convenience.

#### HMBA

Three Phase II trials of this polar-planar differentiating agent in myelodysplastic syndrome and one in malignant melanoma are currently ongoing. The implementation of biologic endpoints cytogenetics, measurement of early- or late- myeloid antigen levels, multilineage bone marrow progenitor cell assays, expression of proto-oncogenes (c-myc, c-fos, c-fms are an intrinsic part of each of these trials. The Phase I oral study (between Walter Reed, CTEP and the intramural program) with comparative pharmacology studies between parenteral formulation and oral formulations (either solution or tablets) has been completed.

### Fludarabine Phosphate (FAMP)

The drug has demonstrated significant single agent activity in alkylator-refractory lymphoproliferative disorders, especially in chronic lymphocytic leukemia (CLL) and favorable histology non-Hodgkins lymphoma. Pilot trials of FAMP-containing combination regimens (FAMP + Prednisone, FAMP + chlorambucil, and FAMP + deoxycoformycin) in CLL are being activated. Application for Group C designation of the drug (for refractory CLL) is underway.

### Merbarone

Phase I trials of Merbarone using either 5-day continuous infusion or 2-hour infusion daily for 5 days are being completed. A number of Phase II trials in a panel of solid tumors are now being planned.

### Dihydrolemerone

This is the first compound chosen for clinical trials based on its activity in the human tumor colony-forming assay; the highest response rate (27%) in this assay was seen in lung cancers. A Phase I trial in lung cancer patients (disease-oriented) is nearly completed at the Navy.

### Chloroquinoxaline Sulfonamide

This is the second compound with outstanding activity in the human tumor cloning assay. The compound is especially active preclinically against melanoma, ovary, breast, and lung tumors. Phase I trials have been started using 1-hour infusion schedule. Based on drug's pharmacokinetic characteristics with the initial schedule of administration, other schedules would be explored.

## DRUG MANAGEMENT AND AUTHORIZATION SECTION

### Drug Accountability

The drug accountability system, implemented in January 1983, has continued to function well. All investigational drugs must be ordered and dispensed to patients by protocol and be documented on an investigational drug accountability form. This has proven to be an essential addition to the site visit monitoring as conducted by the Quality Assurance and Compliance Section. This form has been accepted by several drug companies for use in many of the cancer centers across the country. During the past year we have added a drug transfer form to the NCI Investigational Drug Accountability procedure and have received OMB renewal for the Drug Accountability and drug transfer forms. We have worked closely with the NCI Information Resources Branch to reprint the revised Drug Accountability procedures book. The Drug Accountability form and the drug transfer form have helped to reduce the amount of unused drug which must be returned to NCI.

### Electronic Clinical Drug Request

The section has developed an electronic clinical drug request system for the transmission of drug requests from investigators to NCI. After the system was designed, equipment was purchased and a User's Guide was written. A pilot project was initiated at two major cancer centers: Memorial-Sloan Kettering and M.D. Anderson. The pilot was later expanded to more diverse clinical practice settings, nationwide, bridging several different time zones. These pilot programs have been successful in simplifying the drug ordering procedure and reduced overall drug distribution time from weeks to days. We have begun to offer the Electronic Clinical Drug Request system to investigators, nationwide. The procedure has been well received and has helped to minimize the need for investigators to maintain large drug inventories and it has thus helped reduce drug cost to NCI.

### Computer Costs and Planned System Changes

The DMAS continues to be concerned about the high Computer Costs associated with the current computer system used to monitor the distribution of drugs. In the past year we conducted an analysis of this system. As a result we have received Concept Approval to upgrade it to a state-of-the-art system using a file server, networked PC's and significantly reduce the use of DCRT. Significant enhancements will be added to the system in the coming years which will have of net effect of reduced costs as well as improving system efficiencies and responsiveness to DMAS and CTEP.

### Investigator Registration

The administration of the annual reregistration of investigators continues to be an important function of DMAS. The investigator compliance is presently 100%. Also, during the past year we have completed the implementation the new FDA-1572 forms. Because of the changed format and content of the form and the requirement of more detail, greater interaction with investigators has been required to explain the form changes.

### Drug Cost Expenditures

The total drug costs have decreased from \$ 3.5 million in FY 86 to \$2.9 million in FY 87. Costs have increased as expected in FY 88 to \$ 4.0 million as a result of increased drug distribution to Cooperative Groups, CCOP's, the NCI intramural program and NIAID for AIDS. These increasing trends are continuing in FY 89 and are expected to total \$ 4.5 million.

## Drug Distribution Data for the Past Year

Number of Drug Orders ( <u>Line Items</u> )	Number New Special Exception Protocols ( <u>Reorders</u> )	New Group C Orders	Total Containers (Vials/ampules bottles) <u>Distributed</u>
15,504 (29,904)	696 (778)	550	1,252,986

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## REGULATORY AFFAIRS BRANCH

The Regulatory Affairs Branch is responsible for preparing and submitting Investigational New Drug Applications (INDs) to the Food and Drug Administration (FDA) for assisting in the initiation of clinical trials with anticancer and antiAIDS agents and complying with all FDA regulatory requirements pertaining to these agents. In addition, the Regulatory Affairs Branch implements, coordinates and administers the monitoring of clinical trials with anticancer agents sponsored by the Division of Cancer Treatment, NCI. The Branch assures that clinical trials are conducted according to NIH and NCI policies and procedures and Federal regulations.

The Regulatory Affairs Branch is composed of the Drug Regulatory Affairs Section and the Quality Assurance and Compliance Section. The Drug Regulatory Affairs Section is responsible for:

1. Liaison between the Division of Cancer Treatment, NCI, and both the Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research of the FDA;
2. Submission of INDs to FDA after analyzing the adequacy of the data for cytotoxic and biologic anticancer agents developed by the Division of Cancer Treatment, NCI, and other NCI divisions, particularly the Division of Cancer Biology and Diagnosis;
3. Submission of INDs to FDA after analyzing the adequacy of the data for antiAIDS agents;
4. Coordination of responses to correspondence from FDA regarding IND applications and amendments;
5. Compliance with adverse drug reaction regulations;
6. Liaison with the preclinical sections of the Division of Cancer Treatment, particularly the Developmental Therapeutics Program and the Biological Response Modifiers Program;
7. Liaison with pharmaceutical companies to provide preclinical and clinical data and any other information required to complete approval for New Drug Applications;
8. Liaison with intramural clinical groups in NCI and NIH on regulatory issues concerning agents of particular interest; and
9. Liaison with extramural investigators on regulatory issues concerning agents of particular interest.

The Quality Assurance and Compliance Section is responsible for:

1. Planning, organization and administration of a program for monitoring the quality of clinical data for all clinical trials with anticancer agents sponsored by the Division of Cancer Treatment;

2. Attendance at 10-20% of on-site audits performed by the Cooperative Groups;
3. Carrying out the on-site audits of Cancer Centers and other single institutions conducting clinical research utilizing DCT-sponsored investigational agents;
4. Carrying out special mail and on-site audits of Group C/Treatment Protocols;
5. Carrying out special on-site audits of promising Phase II clinical studies to confirm response rates before decisions are made about future Phase III studies;
6. Serving as the Project Officer for a contract with the Clinical Trials Monitoring Service;
7. Liaison with the Office for Protection from Research Risks (OPRR) and the Cooperative Groups to help new physicians/institutions complete assurances to become able to enter patients on study as rapidly as possible;
8. Setting guidelines for the conduct of DCT-sponsored clinical research and serving as an educational resource to the cancer community for site visit monitoring and regulatory requirements for clinical trials;
9. Review of each protocol submitted to CTEP to assure the informed consent form meets federal guidelines and that other regulatory and policy issues are addressed;
10. Liaison with the Scientific Investigations Branch, FDA; and
11. Performing for-cause audits in response to legitimate patient concerns and complaints or information from outside sources.

The professional staff of the Regulatory Affairs Branch includes the following individuals:

Dale Shoemaker, Ph.D., Chief

Drug Regulatory Affairs Section -

Jay Greenblatt, Ph.D., Head

Maryellen Franko, Ph.D.

Paul Hiranaka, R.Ph.

Quality Assurance and Compliance Section -

Dorothy Macfarlane, M.D., Head

Joan Mauer, B.S., M.T.

Gary Smith, B.S., M.T.

A summary of the activities for FY '89 includes:

1. Thirty-five INDs for cytotoxic and biologic anticancer and antiAIDS agents were prepared and submitted to the Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research of the FDA.

2. The INDs for 10 agents were discontinued.
3. Two Group C applications were submitted to FDA.
4. The data from all adverse drug reactions reported to the FDA since August 1, 1985 have been entered into a data base on a personal computer. Reports were prepared and distributed to the Drug Monitors and other staff in CTEP to help in their evaluation of agent toxicities. During CY '88 279 adverse drug reactions were reported to FDA.
5. On-site audits were made to 19 Cancer Centers or other single institutions which are conducting trials with DCT-sponsored investigational agents.
6. Meetings were held with the Division of Anti-Viral Drug Products of FDA to determine the preclinical data and the IND format required for the agents used to treat patients with AIDS.
7. Procedures were implemented for the monitoring of limited multi-institutional Phase I studies carried out by the Cooperative Groups. Five Cooperative Groups are currently conducting Phase I studies.

#### DRUG REGULATORY AFFAIRS SECTION

##### IND Submissions.

For the FY '89, an Investigational New Drug Application (IND) was submitted to the Center for Drug Evaluation and Research, Food and Drug Administration (FDA), for each of the following compounds:

<u>Drug</u>	<u>NSC Number</u>
Buthionine Sulfoximine	NSC 326321
10-EDAM	Not Assigned
Fostriecin	NSC 339638
Hycamtamine	NSC 609699
Hydrazine Sulfate	NSC 150014
Pentosan	Not Assigned
Porfiromycin	NSC 56410
Pyrazine Diazohydroxide	NSC 361456
Symetamine	NSC 57155
Tetraplatin	NSC 363812
Zidovudine, Acyclovir, DDI and DDC	NSC 602670

INDs were submitted to the Center for Biologics Evaluation and Research, FDA, for the following agents:

<u>Drug</u>	<u>NSC Number</u>
Autologous Tumor Cell Vaccine + BCG and IL-2	NSC 116327
Autologous Tumor Cell Vaccine + BCG and IL-2 (Expanded Lymph Node Cells)	NSC 116327
<u>Drug</u>	<u>NSC Number</u>
GM-CSF (E. coli)	NSC 622183
IL-1 Alpha	NSC 621381
IL-1 Alpha	Not Assigned
Interleukin-2/Pseudomonas Exotoxin (IL-2/PE40)	Not Assigned
IL-3	Not Assigned
Macrophage Colony Stimulating Factor (M-CSF)	NSC 625377
Monoclonal Antibody Chimeric B72.3	NSC 624338
Monoclonal Antibody Chimeric B72.3 Gamma-1	Not Assigned
Monoclonal Antibody Chimeric 14.18	NSC 623408
Murine Monoclonal Antibody B-1	Not Assigned
Murine Monoclonal Antibody CC49	NSC 623111
Murine Monoclonal Antibody COL 6	Not Assigned
Murine Monoclonal Antibody Immu-4	NSC 624344
Murine Monoclonal Antibody Immu-4 F(ab') <sub>2</sub>	NSC 624341
Murine Monoclonal Antibody Immu-14	Not Assigned
Murine Monoclonal Antibody Lym-1	NSC 620858

<u>Drug</u>	<u>NSC Number</u>
Murine Monoclonal Antibody Lym-2	NSC 620859
Murine Monoclonal Antibody 11C64	Not Assigned
Murine Monoclonal Antibody 14G2a	NSC 624345
N2 Transduced TIL	NSC 622283
PEG IL-2	NSC 625376
Xomazyme H65	NSC 620860

#### INDs Discontinued.

INDs for the following agents were discontinued:

<u>Drug</u>	<u>NSC Number</u>
Cisplatin	NSC 119875
Deoxydoxorubicin	NSC 267469
Dianhydrogalactitol	NSC 132313
Diethyldithiocarbamate	NSC 38583
Emofolin Sodium	NSC 139490
Monoclonal Antibody to Human T Cell Antigen (Leu-2a) (Murine)	NSC 370154
<sup>131</sup> I Murine Monoclonal Antibody (45-2D9) to Human Colorectal Carcinoma	NSC 600661
Procarbazine (IV)	NSC 77213
Suramin (AIDS)	NSC 34936
Thymosin Alpha 1	NSC 337793

Group C Applications were submitted to the Center for Drug Evaluation and Research, FDA for each of the following compounds:

<u>Drug</u>	<u>NSC Number</u>
Fludarabine Phosphate	NSC 312887
Levamisole	NSC 177023

The Regulatory Affairs Branch currently maintains 160 active INDs for both cytotoxic and biologic anticancer and antiAIDS agents.

#### Adverse Drug Reaction Reporting.

The Section is responsible for reporting adverse drug reactions to FDA. During CY'88 279 adverse drug reactions were reported to FDA. An additional 676 ADRs were received and processed and held for the Annual Reports to FDA. A package outlining the reporting of adverse drug reactions was mailed to all DCT investigators. The data from these forms are being entered into a data base on a personal computer.

#### Additional Activities.

Revisions were made to the internal procedures for adverse drug reactions (ADRs). Letters continue to be submitted to FDA with whatever summary information we have for ADRs reported by telephone or as a brief communication. A followup submission is made which contains detailed information on the event. This allows the CTEP to better meet the FDA's required reporting timeframes. All ADRs are prepared for review weekly by the Head of the Biologics Evaluation Section and the Head of the Developmental Chemotherapy Section, Investigational Drug Branch. Their review along with that of the Section is essential for determining trends, frequency, etc. Continuing discussions were held with CTEP staff to review suggestions on ways to streamline the ADR process.

Procedures to systematically update Clinical Brochures continue to be implemented, particularly for those agents just entering Phase II trial and for agents of particular interest. The revised Clinical Brochures are provided to all investigators currently using the particular agent.

Guidelines have been developed for the procedures to follow to provide investigational agents to foreign investigators. In addition, development continued on specific guidelines to be used by the CRC/EORTC which will be implemented through Dr. Yoder at the NCI Liaison Office in Brussels, Belgium.

Discussions were held with the CTEP staff, particularly with the Investigational Drug Branch, to determine the tasks to be carried out on the contract for the pharmacokinetic study of anticancer agents.

The Section's professional staff continues to participate in discussions concerning the expansion of the IL-2/LAK cell trials and validate the IL-2/LAK cell process at each new institution. The staff also inspects LAK cell laboratories.



The Section's staff continues to disseminate information and guidelines for the process validation and monitoring of LAK cell generation to all NCI investigators performing human studies with IL-2/LAK, IL-2/TIL and modifications of LAK and TIL cells, i.e. educated LAK and expanded lymph node cells.

The staff prepared the following guidelines to assist extramural and intramural investigators in meeting FDA requirements:

1. Guidelines for Antigen-Specific Immunotherapy (ASI),
2. Revised guidelines for the manufacture and testing of monoclonal antibodies, and
3. Revised guidelines on requirements for IND submissions for IL-2 in combination with adoptive transfer of cytotoxic cells.

The Section's staff reviews all new Biologic Response Modifiers Program monoclonal antibody contracts for compliance with FDA requirements.

The staff advises NCI Monoclonal Antibody contractors on the production, purification and testing of monoclonal antibodies.

The staff worked closely with Dr. W. French Anderson, Dr. Michael Blase, and Dr. Steven A. Rosenberg in getting FDA approval for the first treatment involving retroviral gene insertion into human cells (N2 Transduced TIL).

Procedures for providing preclinical and clinical data to pharmaceutical companies in the most timely manner continue to be implemented. ADRs are sent to the companies at the same time as they are submitted to FDA. Similar procedures are now in place for all protocols approved by CTEP.

The Section's professional staff serves on the Developmental Therapeutics Program Quality Control Committee which reviews and approves certificates of analysis for all biologic and cytotoxic anticancer agents.

The Section's professional staff participated in numerous meetings with pharmaceutical companies to outline the Branch's operating procedures and explain its role in CTEP's drug development process. In addition, a document which outlines the roles to be carried out by CTEP and by the pharmaceutical company for co-development of an agent is being developed.

#### QUALITY ASSURANCE AND COMPLIANCE SECTION

The Quality Assurance and Compliance Section is responsible for on-site monitoring of all clinical trials sponsored by the Division of Cancer Treatment. This includes all trials conducted by the Cooperative Groups, and studies conducted at Cancer Centers or other individual institutions which utilize DCT-sponsored investigational agents.

### Cooperative Group Site Visits.

In the case of the Cooperative Groups, DCT has delegated the responsibility for organizing and conducting the monitoring program to each group. Each institution is to be monitored at least once every three years. During the past year, the Cooperative Groups site visited 108 member institutions, 183 affiliates and 33 CCOPs (or CCOP components).

The Quality Assurance and Compliance Section continues to co-site visit with the Cooperative Groups in 10-20% of the scheduled visits to assure the adequacy of the audit procedures. In addition, the Cooperative Groups submit a report on each on-site audit to the Section for review.

New guidelines were implemented for the monitoring of limited multi-institutional Phase I trials conducted by Cooperative Groups. Five Cooperative Groups are presently conducting Phase I studies.

### Phase I and Single Institution Site Visits.

The Quality Assurance and Compliance Section directly oversees the monitoring of Phase I and Cancer Center studies. Phase I studies are monitored three times per year. During the past year, 19 visits to Cancer Centers or other single institutions conducting trials with DCT-sponsored investigational agents were accomplished.

### Additional Activities.

Four special audits were carried out to examine the data and verify response determinations in promising Phase II trials. These included: Dibromodulcitol in ovarian cancer, Suramin in prostate cancer, high dose Carboplatin in ovarian cancer and Alpha IFN/5-FU in colon cancer.

The adverse drug reaction (ADR) reporting from Cooperative Groups and other investigators using DCT-sponsored investigational agents is monitored closely. A standard ADR reporting section is required in each protocol.

The New Drug Study Group application is included with the LOI approval letter for any institution wishing to do independent studies which is not an NCI-supported Cancer Center.

Applications are reviewed and approved by Section staff in cooperation with Investigational Drug Branch staff.

All protocols submitted to CTEP were reviewed to determine whether they would be conducted as single institution or multicenter trials, and to assure that the conduct of multicenter trials would meet quality assurance guidelines.

An audit results database for Cooperative Groups is maintained and includes results of all audits conducted since January 1985.

This Section is also responsible for setting guidelines and standards for the conduct of clinical trials in order to assure data quality and compliance with regulatory requirements for clinical research. In addition, the Protocol and Information Office (PIO) was transferred from the Office of the Associate Director, CTEP to the Quality Assurance and Compliance Section, RAB. The PIO is responsible for the protocol review process as well as serving as the receipt point at NCI for all protocols entered into the PDQ system.



## SUMMARY REPORT

### ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM

#### DIVISION OF CANCER TREATMENT

#### NATIONAL CANCER INSTITUTE

October 1, 1988 - September 30, 1989

The Clinical Oncology Program (COP) is the intramural treatment research arm of the National Cancer Institute. The Program, which is comprised of six Branches, conducts basic and clinical research in medicine, pediatrics, surgery, pharmacology, radiobiology, endocrinology, immunology, genetics, and molecular biology in the context of developing curative therapies for cancer. A laboratory under the supervision of Dr. Samuel Broder operates under the auspices of the Office of the Associate Director (OAD). This Office supports a Biostatistics Data Management Section, supervised by Dr. Seth Steinberg.

#### PROGRAM ACCOMPLISHMENTS

##### OFFICE OF THE ASSOCIATE DIRECTOR

##### Laboratory Investigator-initiated Research Activities

The OAD virtually reprogrammed all its scholarly activities in response to the AIDS epidemic and to the Department's focus on AIDS as a number one priority. A portion of Dr. Broder's investigator-initiated research activities is summarized under the following heading:

##### Development of Anti-retroviral Agents for the Therapy of AIDS and Its Related Diseases:

AIDS and AIDS-related diseases are caused by the third known pathogenic human retrovirus, now called human immunodeficiency virus (HIV). During the past year, Dr. Broder's laboratory has continued to develop technology for the rapid detection of drugs or biologics which can suppress the replication of HIV in vitro. In addition, his group has continued its efforts to bring promising drugs to clinical trials as quickly as possible. At this time, it is possible to say that the general scientific perspective on the development of anti-retroviral drugs has changed: the question no longer is whether clinically active drugs can be developed for the treatment of AIDS, but how many agents will be found and how best to prioritize development of these agents.

A more complete discussion of various agents and therapeutic strategies will be taken up in the laboratory project report section. In summary, Dr. Broder's laboratory has focused on the development of certain anti-retroviral nucleoside analogues, their biochemical pharmacology, and their application to the therapy of patients with AIDS and related disorders. In addition, certain targeted therapies designed to inhibit HIV binding to cells or to the suppression at the genomic level have been explored.

One family of anti-retroviral nucleosides is referred to as dideoxynucleosides. The first in vitro assessment of these drugs against HIV was undertaken in this laboratory about five years ago and has been discussed in previous annual reports. About four years ago, one member of this family, AZT, was used by COP for the first time to treat patients with AIDS. This drug is the first and, at present, the only anti-retroviral chemotherapeutic agent approved for prescription status.

During the past year, new studies of AZT have been undertaken. One of the primary toxicities of AZT is bone marrow suppression. In an attempt to obviate this problem, a feasibility study was initiated to test whether AZT's bone marrow suppressive activity could be ameliorated by the administration of GM-CSF. GM-CSF is a bone marrow stimulant that can promote the regeneration of cells belonging to the granulocyte and macrophage series.

In this first study of its kind, AZT was given in an alternating manner with GM-CSF to patients with AIDS. Ten patients have been studied extensively. The results suggest: (1) that GM-CSF can potentiate bone marrow function in AIDS patients and (2) that an alternating regimen of GM-CSF and AZT is clinically active in suppressing viral replication, permitting increased T4 counts and possibly better tolerance to an AZT-related form of bone marrow suppression.

At the same time, these studies showed that GM-CSF, if given by itself (that is, without AZT), actually could increase the replication of HIV in patients with AIDS. This clinical finding is in accordance with the laboratory finding made by this laboratory that GM-CSF can enhance HIV replication in human monocytes. However, as will be discussed below, GM-CSF can increase the anti-HIV activity of AZT in vitro in such cells. A small-scale clinical study of the simultaneous administration of AZT and GM-CSF is now underway in the Program.

The Program also has continued a feasibility study of AZT alternating with dideoxycytidine (ddC), another dideoxynucleoside, in patients with AIDS or AIDS-related complex (ARC). In the last year, a phase I study of ddC was completed. This drug was shown to exert a virustatic effect in vivo in patients with AIDS and related disorders. The major dose-limiting toxicity was not bone marrow suppression (as with AZT), but rather a peripheral neuropathy. AZT does cause peripheral neuropathy. Because of these non-overlapping toxicities, a pilot study testing a regimen of weekly AZT, alternating with weekly ddC, was initiated. This regimen was found to be clinically active.

Some patients have been enrolled in this regimen for more than two years without significant toxicities. The combination of AZT and ddC seems to have profoundly reduced the level of bone marrow suppression observed with the administration of only oral AZT. The risk of peripheral neuropathy associated with single-agent ddC at high-dose continuous administration also seems to have been reduced significantly. The patients who developed a peripheral neuropathy usually had a mild, reversible form. Generally, the peripheral neuropathy did not occur during the first six months of the regimen. These results have led to the initiation of a large-scale, multi-center study to determine whether an alternating regimen of AZT and ddC is more active and less toxic than single-agent therapy.

During the past year, studies of two related dideoxypurines, dideoxyadenosine (ddA) and dideoxyinosine (ddI) were initiated. These drugs were shown to be

potent inhibitors of HIV replication, but exerting little toxicity to T cells. ddA is rapidly converted to ddI by the ubiquitous enzyme adenosine deaminase, and, for many purposes, both may be considered alternate forms of the same drug. Both drugs are cleaved into dideoxyribose and the free base under acid conditions (as in the stomach). However, while adenine, the free base of ddA, can cause renal damage, the free base of ddI, hypoxanthine, is relatively well handled by the body. For this reason, ddI is probably the preferred form for oral administration.

A small study of ddA given by intravenous administration showed that the drug was well tolerated and could induce an increase in T4 cells and a decrease in HIV p24 antigenemia in patients with AIDS or AIDS-related complex. In addition, we documented that the drug almost instantaneously was converted to ddI in the body. Subsequently, we have initiated a study of ddI, first given by the intravenous route, then later by the oral route. When given with antacids, the drug was found to have a 30% to 40% oral bioavailability and to penetrate the central nervous system.

At the lowest dose of ddI tested (0.2mg/kg intravenously every 12 hrs, then 0.4 mg/kg every 12 hrs or higher), almost every patient had increases in their T4 cells and, if originally positive, a decrease in their viral load (as measured by HIV p24 antigen). In addition, several patients showed improvement in immunologic function (as assessed by delayed-type cutaneous hypersensitivity or in vitro T cell proliferative responses). Finally, the patients had evidence of clinical improvement as assessed by increased energy, increased appetite, and an average 1.6 kg weight gain.

So far, the toxicity has been minimal: some patients at the highest doses had 1 to 3 mg/dl increases in uric acid, several patients had headaches or insomnia, two patients had seizures, one patient had an increase in amylase, and one patient had dysesthesia of the feet. Some patients have now been on ddI for over 40 weeks without toxicity. In addition, five of the patients originally started on ddA subsequently were switched to ddI; some of those patients have now tolerated ddA or ddI for over a year. So far, ddI has one of the most favorable toxicity profiles of any anti-AIDS drug studies. Large controlled Phase II trials of ddI are now in the planning stage.

In collaboration with Genentech, Inc., a study of recombinant CD4 in patients with AIDS was conducted, perhaps representing the first time that a specifically-designed, targeted therapy has been used in the treatment of AIDS. CD4 is a glycoprotein on the surface of T cells and certain other cells and acts as the receptor for HIV. Recombinant CD4 (rCD4) is a glycoprotein produced by genetic engineering technology which contains the extracellular domains of CD4. It can bind to HIV and, therefore, block its binding to, and infection of, human lymphocytes and monocytes. The rCD4 clinical study involved continuous infusion to patients with severe HIV infection at doses up to 1000/ug/kg/day. The half life of rCD4 is about 40 minutes. Essentially no drug toxicity was observed, and no patient developed anti-CD4 antibodies. Some patients had decreases in their HIV p24 antigen or increases in their T4 cells, but this was not consistently observed. It is still not clear if an anti-HIV effect is observed in patients given rCD4.

At the same time, in vitro studies to explore new hybrid compounds of CD4 and immunoglobulins have been conducted in collaboration with Genentech. CD4 is in

the same supergene family, and hybrid proteins combining the first two domains of CD4 (which bind to gp120) with the Fc portion of IgG heavy chain retain certain desirable properties of both moieties. In particular, such hybrid proteins bind avidly to gp120 and, at the same time, will stay in the circulation much longer than rCD4. In addition, it may preserve certain immunologic functions of immunoglobulin. The studies in the laboratory showed that these proteins effectively inhibited the HIV infection of monocytes. Clinical trials of a hybrid CD4-immunoglobulin protein are expected to start in the near future. Other laboratory studies have revealed that CD4 inhibits HIV-1 infection better than it inhibits HIV-2 infection. Studies are underway to determine the molecular basis for these differences.

It has been proposed that, since monocytes have low levels of kinases, dideoxynucleosides may have a limited capacity to inhibit HIV replication in such cells. Some reports suggested that macrophages provided a reservoir for HIV replication in such a way that various drugs could not affect their replication. During the past year, a system for studying anti-retroviral drugs in macrophages was established. The results provided strong evidence that all the dideoxynucleosides studied (including AZT) could block HIV replication in macrophages. However, the reasons for this were unexpected. Macrophages were found to contain low levels of kinases (activating enzymes), necessary to convert the various drugs into phosphorylated derivatives which actually suppress viral replication in the cell.

Nevertheless, in collaborative studies with Dr. David Johns of the Developmental Therapeutics Program, the normal phosphorylated nucleotides, which corresponded to the dideoxynucleoside drugs, were extremely low in macrophages compared to T cells. The low normal nucleotides meant that there was less competition for the anti-retroviral effects of the drugs against the virus at the level of its reverse transcriptase. As a continuation of these studies, it was determined that, while GM-CSF enhances HIV replication in monocytes, it increases the entry of AZT into such cells and its phosphorylation so that AZT is effective at lower doses than in the absence of GM-CSF. Clinical studies exploring the combined simultaneous use of AZT and GM-CSF are now underway.

During the past year, a number of structure-activity relationships involving nucleoside analogues have been explored. For example, an epoxy congener of cytidine has been found to possess considerable activity. This drug, 1-(2',3'-anhydro-beta-D-lyxofuranosyl)cytosine, was prepared in collaboration with Dr. Tom Webb at Genentech, Inc. It is one of the few epoxy analogues known to possess potent anti-retroviral activity. Also, during the past year, in collaboration with Dr. Jiri Zemlicka at the Michigan Cancer Foundation, a new class of acyclic compound activity against HIV-1 and HIV-2 was discovered. Adenallene and cytallene, represent two newly-synthesized compounds. A study of structural activity relationships revealed that the presence of two cumulated double bonds between the 2' and 3'-carbons conferred anti-retroviral activity in certain pyrimidine and purine derivatives containing a four-carbon side chain. These compounds are now being evaluated for possible further development.

A special effort has been made to identify new categories of drugs or biologics which could attack HIV at different steps in its life cycle. A great deal of attention has been focused on polyanionic polysaccharides. One representative of this class of compounds is dextran sulfate. We have observed that this agent is a potent inhibitor of HIV replication in vitro and that at least one



mechanism for the observed activity is a capacity to block virion binding at the surface of target cells. Other agents which block virus binding to the cell surface are now being studied.

Finally, a major effort has been underway to deal with viral replication and expression in cells that are already infected. Virtually all the major drugs, which now are used in clinical studies, affect viral replication by protecting uninfected target cells from becoming infected by HIV. In collaboration with Dr. Jack Cohen's laboratory, certain novel oligodeoxynucleotides have been studied for capacity to inhibit viral replication. Special attention has been focused on phosphorothioate analogues, compounds in which one of the non-bridging oxygens has been replaced by a sulfur atom. Such constructs are quite stable and resistant to enzymatic attack. These compounds have a sequence-specific and non-specific activity. The sequence non-specific activity appears to be mediated by a competitive inhibition of template-primer interactions within the viral reverse transcriptase.

During the past year, we have focused on the sequence-specific inhibition. The mechanism appears to be translation arrest. We have observed that a 28-mer phosphorothioate analogue of an oligodeoxynucleotide, which is in an anti-sense configuration to the art/trs gene of HIV, can block the expression of virus even in chronically infected target cells. Full length (genomic) viral mRNA seems to undergo the greatest reduction in infected cells that are exposed to this construct. Envelope expression is affected, but to a lesser extent, while smaller transcripts such as 3'orf seem to be unaffected. This may provide the first proof that one can affect viral expression in a chronically infected target cell without necessarily killing the cell. A continuation of this study has shown that combining intercalating compounds with such antisense constructs selectively can increase their anti-HIV activity.

With some tools at hand, the Program has tried to make an impact on AIDS and the retrovirus which causes this disease. Many of these tools were discovered or identified in Program research performed in previous fiscal years. At the same time, the Program has embarked on a campaign to find new tools for future interventions against this disease.

#### Biostatistics and Data Management Section

BDMS is the statistical and data management component of the COP. The Section provides statistical leadership and data management consultation for the Program's major activities. It is involved in the design, conduct, monitoring, and statistical analyses of intramural and national multicenter clinical trials for experimental cancer treatments. Other major collaborative efforts include studies to identify important prognostic and treatment selection factors; to evaluate diagnostic procedures; to develop improved staging systems; and to assist investigators in the design, execution, and analyses of major in vitro drug testing.

BDMS develops new statistical designs and biometric methods related to the development and evaluation of new cancer treatments. The Section maintains computerized data collection systems for intramural and national multicenter clinical protocols; works closely with interested branches to improve data recording and retrieval; and is working to develop specialized clinical data bases for the COP branches.

The Section works with the Clinical Center Medical Information System (MIS) team, enabling Program input for decisions directly impacting patient care and protocol management. The Section assists the NCI Deputy Clinical Director with proper monitoring of protocols through the MIS Toxicity screens and other mechanisms.

### MEDICINE BRANCH

This year has been one of large-scale change with a turn-over in 80% of the clinical staff, 50% of the research nurses and most of the technicians. The Medicine and Clinical Pharmacology Branches have merged, providing an opportunity for massive restructuring of the Branch. Despite these changes, the Branch has been quite productive in numbers of published papers and in importance of the clinical observations.

### Changes in Branch Structure

The Branch initially was designed to supervise 4 fellows and one 22-bed ward. As of last summer, the Branch became responsible for 12 fellows and 78 beds. This expansion created the need for a sufficient infrastructure to manage this large clinical resource. A protocol office with research nurses, data managers, a secretary and a clinical care coordinator was established to deal with all patient-related issues including accepting new patients.

This office will supervise the entire process of protocol design and approval; once a protocol begins, data management, FDA and CTEP reporting and monitoring the progress of ongoing trials will be an integral part of the office operation. To streamline the process of clinical data management, outside contractors were consulted to design data base software. This base is almost complete and will be used for all our trials.

The second major change has been dictated by the need to reduce clinical center costs and to restructure the operation of in- and out-patient clinics. In the past, the Branch optimized the use of the in-patient beds at the expense of the out-patient service. Because it is critical to move as much care as possible to the out-patient department, we needed to assess this issue. The previous set-up left the clinic space unused for most of the morning. The new schedule, which will allow the clinic to function all day at full capacity, hopefully will effect an efficient utilization of the clinic space. We also have initiated disease-specific clinics that will be manned by the protocol principal investigators. Patient-related questions will be answered more quickly, increasing the efficiency and goodwill of the clinics.

### Clinical Advances

Suramin. While we have worked on suramin for 4 years, the past year has been particularly rewarding in the development of this drug. We have been able to put 35 patients with prostate cancer on this drug. Preliminary results in patients with soft tissue disease suggest that the overall response rate will be 40-60% in patients who have failed previous hormonal therapy. If these results prevail, suramin will be the most active drug in the treatment of this common cancer. Suramin also has shown significant activity against heavily pre-treated lymphoma patients.

GM-CSF + CBDCA in the Treatment of Refractory Ovarian Cancer. In this trial, we attempted to use GM-CSF to lessen the bone marrow toxicity of CBDCA, allowing for higher dosages. Because ovarian cancer has a steep dose response curve for platinum-containing drugs, we anticipated that this might lead to an increased response rate. Patients in this trial had to progress through frontline chemotherapy. Thus far, the response rate is 50%. This percentage is better than any other result in a comparable treatment population: high dose cis-DDP gave approximately 30% response rate in this setting.

Potential Molecular Basis for Adrenal Carcinoma. Demonstration of high IGF-II expression in 8/8 human adrenal carcinoma biopsies and little or no expression in normal human adrenal tissue, suggests an important role for this growth factor in the pathogenesis of adrenal carcinoma.

Basis for Cis-DDP Responsiveness. In collaboration with Dr. Muggia at USC, treatment of ovarian cancer with single agent cis-ddp revealed an association between leukocyte platinum-DNA adduct level and subsequent response to therapy. In fact, only the adduct level at the end of the first cycle may be sufficient to distinguish responders from non-responders. Collectively, these data indicate that variation in the expression of DNA repair enzymes within the patient population may be the major determinant of drug sensitivity.

### Laboratory Advances

1. Discovery that interferon reversed 5-Fu resistance by blocking over-expression of thymidylate synthetase.
2. Discovery that a metabolite of folate, 10-formyl-dihydrofolate, is a potent inhibitor of thymidylate synthetase and GAR transformylase.
3. Unexpected discovery that the sulfones appear to be unusually effective inhibitors of *I. gondii* dihydrofolate reductase and have considerable promise in the treatment of this infection in AIDS patients.
4. Demonstration that *P. carinii* is more closely allied with fungi than protozoans. This demonstration was done through analysis of ribosomal RNA sequences, an expected means of establishing taxonomy.
5. Cloning of the gene for a putative folate transporter gene from MTX resistant cells.
6. Demonstration that the human GST pi gene has a consensus sequence similar to the binding site for AP-1 transcription factors, which include the c-jun oncogene. In turn, Fos has been shown to interact with Fos to increase further expression of AP-1 regulated sites. These results may explain why GST-Pi is found selectively increased in expression in tumors versus normal tissue (in colon, for example, where GST-Pi expression makes an excellent correlation with malignant transformation). This work has recently been published in Gene.
7. Demonstration that, in breast cancer, the development of multidrug resistance is associated with the loss of estrogen receptors and a marked increase in EGF-receptor expression.

8. Demonstration of marked heterogeneity of sis oncogene expression in human gliomas with sis expression correlating with a more differentiated phenotype. Sis encodes for the expression of PDGF and sensitivity of human gliomas to suramin, a PDGF antagonist, correlates well with sensitivity to this drug. These results may pave the way for a clinical trial testing the effectiveness of suramin in sis positive and negative gliomas.

9. Identification of the mechanism of uptake of oligonucleotides by cells and the basic structural requirements for uptake.

10. Demonstration that antisense compounds can inhibit N-myc expression in neuroblastoma cells along with a effecting proliferation.

11. Human excision nuclease, ERCC1, has been identified which confers resistance to cis-ddp. Preliminary data suggest that expression of this particular gene may be the important variable in sensitivity of ovarian cancer and normal tissues to cis-ddp.

#### NCI-NAVY MEDICAL ONCOLOGY BRANCH

#### Tumor Suppressor Genes (Recessive Oncogenes) in Lung Cancer

Lung cancer cells over the past 2 years have been found to exhibit genetic abnormalities of many potential recessive oncogenes. These ongoing studies have identified abnormalities in the rb gene (chromosome region 13q14) in potentially all small cell lung cancers and several non-small cell lung cancers. Most recently, small cell lung cancer mutants of the rb gene have been described that give aberrant protein forms. (In collaboration with Drs. Horowitz and Weinberg, MIT)

Recent studies have identified p53 (chromosome region 17p13) as a major recessive oncogene in over 50% (and probably a much greater fraction) of all types of lung cancers.

Chromosome and restriction fragment length polymorphism studies have identified lesions on multiple other chromosomes including chromosome regions 3p, 1p, 1q, 5q, 11p, and 22. Because such tumor suppressor genes usually require inactivation of both the maternal and paternal chromosomes this would indicate that as many as 10-15 different genetic lesions have occurred in clinically evident lung cancer. These results have direct bearing on future prevention and prognostic studies and direct the search for early molecular detection of lung cancer and/or the detection of patients exhibiting some of these abnormalities in a premalignant phase. Studies are ongoing to try to "correct" malignancy in lung cancer cells by reintroducing the suppressor genes into lung cancer cells through transfection and retroviral vectors. (Minna, Kaye, Birrer, Takahashi, Rosenberg, Johnson, Gazdar in collaboration with J. Whang-Peng)

#### Dominantly Acting Oncogenes

The role of transcription factors in lung cancer cells including members of the jun family (c-jun-A, Jun-B, Jun-D), as well as proto-oncogene Fos. We have found deregulated expression of some jun members with loss of control of normal regulatory signals (such as serum and phorbol esters). In contrast, no mutations in the coding regions of the genes have been noted. We demonstrated

for the first time, that deregulated expression of normal jun members in concert with a mutated ras gene can transform normal rat embryo cells to malignancy. Because of the relationship of these transcription factors to stimulation by tumor promoters, these findings create the scenario where normal lung and lung cancer cells appear to be in a state similar to chronic tumor promotion. (Minna, Schütte, Birrer, Viallet, Segal)

A detailed structure function analysis of the c-jun-A oncogene has been undertaken and demonstrates a striking correlation between transforming activity and amino acid sequence conservation between jun family members. (Birrer, Alani [HHMI Research Scholar])

Retroviral vectors and transfection techniques have been developed for delivering tumor suppressor genes and dominant negative oncogenes into cancer cells as well as for use in delivering oncogenes into normal bronchial epithelial cells to study carcinogenesis. (Birrer, Rosenberg)

Specific DNA sequences in the promoter region that regulate transcription positively or negatively have been identified for the L-myc oncogene. These include a new 8bp palindrome present in the 5' region of several other genes. Since there is loss of negative regulation in this area of myc family members in human cancer these studies provide a new approach to understanding the loss of control of oncogene expression in human malignancy. (Kaye, Barksdale [HHMI Research Scholar])

Structural analysis of the L-myc protein and its translation using in vitro mutagenesis has identified a non-AUG initiation codon for one of the protein forms, and phosphorylation changes for the other forms. (Birrer, Dosaka)

Deregulated expression of the L-myc gene, although not expressed in mouse erythroleukemia cells, can substitute for c-myc for blocking differentiation. (Segal, Birrer)

The normal myb genes was shown to block erythroid differentiation, and that the late, but not the early, down-regulation of both myc and myb is required for terminal differentiation. These studies provide the foundation for understanding the molecular defects in differentiation that occur in tumor cells. (Kuehl, Timblin)

We have developed a general method for subtractive cloning incorporating polymerase chain reaction (PCR) technology into the preparation and analysis of subtractive cDNA libraries. This was used to isolate new candidate genes which are expressed uniquely in murine plasmacytoma cells. These techniques are being used to study human B-cell malignancies and understand molecular defects in differentiation in tumor cells. (Kuehl, Timblin, Bergsagel)

Transfection of a c-myc cDNA into an IL3-dependent murine pregranulocyte leukemia cell line results in cells which have increased survival in medium containing G-CSF without IL3, but have lost the ability to terminally differentiate in this medium. (Foss, Kuehl)

Hematopoietic cell lines have been initiated from the bone marrows of several patients with mycosis fungoides. Preliminary analysis suggests that these cells have a non-lymphoid (?stromal cell, ?monocytoid cell) phenotype. Preliminary

results indicate that the medium from one of these lines contains reverse transcriptase activity, suggesting the presence of a retrovirus. (Foss)

Study of Peptide Hormones, Growth Factors, Receptors, Signal Transduction Pathways, and Other Markers Important in the Pathogenesis of Lung and Other Human Cancers

An insulin like growth factor (most similar to IGF-1) has been found to function as an autocrine growth factor for all types of lung cancer and colon cancer, providing a new potential target for anti-tumor therapy and diagnosis. (Cuttitta, Mulshine)

GRP gene related peptides (GGAPs) have been identified and characterized in lung cancer cell lines and tumor specimens. Specific receptors for some of these peptides have been discovered and they have been found to stimulate the growth of lung cancer cells in vitro providing another target for diagnosis and therapy. (Cuttitta, Mulshine, Linnoila)

Receptors for the major addictive agents opioids and nicotine were found on all types of lung cancer. The opioids were found to negatively influence lung cancer growth in vitro while nicotine, in many cases, reversed this effect. These findings provide new approaches to therapy and suggest focusing a major effort on the role of nicotine not only as an addictive agent in smoking, but as possibly playing a major role in the pathogenesis of lung cancer. (Minna, Manackjee)

Atrial natriuretic factor (ANF) was found to be produced by many small cell lung cancers and is a candidate for inducing hyponatremia in lung cancer patients. ANF now becomes another target for diagnosis and therapy. (Johnson, Brennan)

Cholera toxin was found to dramatically inhibit the in vitro growth of many lung cancer cell lines. This was related to the expression of specific glycolipid membrane receptors. Further preclinical studies of this toxin are underway to attempt to bring it to clinical trial in the treatment of lung cancer. (Viallet, Minna, in collaboration with E. Sausville, now at the Lombardi Cancer Center)

Studies of drug resistance and sensitivity in lung and colon cancer cell lines has revealed: 1) excellent correlation between in vitro chemosensitivity and resistance and response in patients; 2) that the MDR1 gene does not appear to play a role in multiple drug resistance in lung cancer; and 3) provides the first good evidence that no true synergism exists between etoposide and cis-platin in the response of lung cancer. (Gazdar, Kramer, Lai, in collaboration with Goldstein and Gottesman, DCBD)

Monoclonal antibodies developed against lung cancer have shown dramatic ability in early detection studies. These have led to the design and implementation of prospective cooperative studies (with Johns Hopkins, and the Lung Cancer Study Group) to validate these findings. The results could have potentially tremendous impact on the early diagnosis of lung cancer. (Mulshine, in collaboration with Johns Hopkins Investigators and the Lung Cancer Study Group)

Immunohistochemical detection of the SAP-35 protein (surfactant associated protein, 35 kd) has been shown to identify a much higher than previously

recognized number of non-small cell lung cancers with bronchoalveolar properties. These findings have important implications for studying the epidemiology of lung cancer in the United States. (Linnoila, Gazdar, in collaboration with the Lung Cancer Study Group, and Eastern Cooperative Oncology Group)

Synaptophysin has been discovered to be a new neuroendocrine marker of lung cancer differentiation. The expression of such neuroendocrine markers has been found to correlate with drug sensitivity and potentially with prognosis. (Linnoila, Mulshine, Gazdar, in collaboration with the Lung Cancer Study Group and the Eastern Cooperative Oncology Group)

### Initiation of AIDS-related Studies

Evidence for a potential new retrovirus in patients with T cell lymphomas, separate from HTLV-1 and HIV, has come from newly-established cultured cells from patients with cutaneous T-cell lymphomas. This virus is being molecularly cloned and characterized. (Foss)

Search for retroviruses related to HIV as a causative agent in human bronchoalveolar and other types of lung cancer and attempts to clone and characterize the retrovirus causing lung cancer in sheep. These include epidemiologic studies in collaboration with the Epidemiology Branch (Madigan and Mulvihill), as well as pathologic review of lung cancer incidence and the changing epidemiology of bronchoalveolar lung cancer. (Minna, Osborne, Linnoila, Gazdar)

Development of clinical tests of drugs inhibiting HIV replication as a treatment for human cutaneous T-cell lymphomas. With evidence for retrovirus infection in cutaneous T cell lymphomas and their role as a possible stimulation for the development of T-cell proliferation, we are developing protocols to test new anti-HIV drugs in this disease. (Foss, Kramer)

### Chromosomal Rearrangements during Normal and Malignant Hematopoiesis

A new gene, scl, has been found at the site of a reciprocal translocation (t(1;14)) from the malignant cells from a patient with a stem cell leukemia. The translocation has resulted in a truncated transcript of this gene fused to sequences within the T cell receptor alpha chain locus. A normal transcript has also been identified with a spectrum of expression consistent with its involvement in hematopoietic stem cell growth and development. (Kirsch, Begley, Aplan)

A fusion gene between two T cell receptor chains, beta and gamma, has been cloned from a cell line and the original peripheral blood derived from a patient with ataxia-telangiectasia. This fusion can explain the morphologic chromosomal abnormality, inv(7), observed in this patient and which appears characteristic of ataxia telangiectasia. Of great interest, the same abnormality occurs at a much lower frequency in normal individuals. (Kirsch, Stern, Lipkowitz)

A characteristic abnormality of chromosome 14 highly associated with adult T cell prolymphocytic leukemia has been cloned from the chronic lymphocytic leukemia cells of a patient with ataxia-telangiectasia and another patient with T cell acute lymphoblastic leukemia. The genes involved in this translocation

are likely to be important to the development of T cell malignancies. (Kirsch, Davey, Bertness)

DNA and RNA from lymphomas and leukemias have been characterized for immunoglobulin and T cell receptor loci to provide unique molecular "fingerprints" for following tumor cell clones in these patients. These have shown distinct patterns reflecting target cell maturation for these tumors. In addition, techniques for RNA in situ hybridization and direct RNA sequencing from patient samples have been developed which will allow a molecular pathologic characterization of lymphomas and leukemias. (Felix, Seibel, Kirsch, in collaboration with the Pediatric Oncology Branch)

#### Major Staff and Administrative Changes in FY 1989

Dr. Daniel C. Ihde, Deputy Branch Chief and Head of the Division of Hematology/Oncology for the Uniformed Services University of the Health Sciences has been appointed as the new Editor-in-Chief of the Journal of the National Cancer Institute.

Dr. Barnett Kramer has been appointed Head of the Medical Staff Fellow training program (in Medical Oncology) for NCI.

Dr. James L. Mulshine has been appointed Head of the newly-established Biotherapy Section of the NCI-Navy Medical Oncology Branch.

Dr. Ilan Kirsch has been appointed Head of the newly-established Acquired Gene Rearrangements Section for the NCI-Navy Medical Oncology Branch.

Dr. John Minna was appointed as the Head of the newly-created Oncogene Advisory Committee for NCI.

Dr. Shoshana Segal has been promoted to Associate Professor of the Department of Medicine at the Uniformed Services University of the Health Sciences.

Dr. Frank Cuttitta has been promoted to Associate Professor of the Department of Medicine at the Uniformed Services University of the Health Sciences.

Sarah Barksdale and Rhoda Alani, two members of the Howard Hughes Medical Institute Research Scholars Program, completed their year of research training.

#### PEDIATRIC BRANCH

##### Clinical Studies

1. Newly diagnosed patients with acute lymphoblastic leukemia:  
NCI 77.02-CCG 191 - This protocol tested, in a randomized study, whether central nervous system preventive therapy using systemic high-dose methotrexate infusions alone (without cranial radiation) is equally effective and less toxic than 2400 cGy of cranial radiation and intrathecal methotrexate. 181 patients were randomized on this study. The overall remission rate was 98% with an event free survival of approximately 70% at three years for the entire study group. With a median duration on study of 76 months, there is no significant difference in the CNS relapse rate for either treatment group. Long-term follow-up evaluation of neurotoxicity (by CT scan, neuroendocrine evaluation and



psychometric testing) is in progress. A recent analysis of patients longitudinally assessed with a periodic neuropsychological test battery demonstrated a striking decrease in verbal and full scale IQ in patients treated with cranial radiation and intrathecal chemotherapy. In addition, patients treated with cranial radiation and intrathecal therapy manifested significant impairment of academic achievement. No such declines were seen in the high-dose methotrexate groups. These data thus indicate that use of combined cranial radiation and intrathecal therapy can be avoided in nearly 60% of children with ALL, reducing the potential long-term neurotoxicity associated with such combined therapy. In contrast, this study has demonstrated no apparent adverse effects of high-dose methotrexate on cognitive functioning and academic achievement, confirming the value of high-dose methotrexate as central nervous system preventive therapy for children with ALL.

NCI 83P-CCG 134P - Treatment of newly diagnosed acute lymphoblastic leukemia in high-risk patients. The major aim of this study is to demonstrate that high-risk patients can be effectively treated on a regimen which uses CNS preventive therapy devoid of cranial radiation. An additional objective is to determine whether there is a difference in the outcome of patients at high risk for early treatment failure according to whether they do or do not have features consistent with "lymphoma leukemia syndrome." The protocol involves the use of an aggressive, early intensification phase of therapy and intensive systemic maintenance therapy, together with CNS specific treatment. The latter consists of periodic administration of systemic high-dose methotrexate, systemic high-dose cytosine arabinoside and intrathecal cytosine arabinoside and methotrexate. With a median potential duration on study of 3.4 years, the event-free survival is projected at 68 percent at two years. The occurrence of isolated CNS relapse in only two of the 102 patients enrolled in this study to date, indicates that this study has been successful in demonstrating effective central nervous system preventive therapy can be achieved in high-risk patients without the use of cranial radiation.

NCI 84A-CCG 144 - This protocol treats newly diagnosed patients in the "average-risk" category, randomizing them to one of two forms of CNS preventive therapy, either high-dose systemic methotrexate infusion or intrathecal methotrexate alone. The median potential duration on study is 30 months. A total of 176 patients have been randomized. There is no significant difference in either the CNS or bone marrow relapse rate in either treatment arm. At the present time the event-free survival at 24 months is approximately 80 percent. These data demonstrate that average-risk patients can receive effective CNS preventive therapy with intrathecal methotrexate alone and do not require high-dose methotrexate.

2. Intrathecal Diaziquone (AZQ) - A new clinically useful agent for the treatment of meningeal neoplasia--AZQ is a lipid soluble aziridiny benzoquinone designed for enhanced CNS penetration of the CNS to treat CNS neoplasms. Despite evidence of clinical activity demonstrated in Phase I and Phase II trials, systemic administration has been limited by severe and prolonged myelosuppression. To circumvent this problem, we are evaluating the feasibility of intrathecal AZQ in a Phase I-II trial in patients with refractory meningeal malignancy. Two schedules of administration are being examined: twice a week for four weeks and "CxT", every 6 hours for three doses, weekly x 4. A total of 5 patients have been treated, 26 of whom had acute lymphoblastic leukemia. Demonstrable antineoplastic activity has been observed on both schedules of

administration. 15 of the 34 courses delivered on the twice weekly schedule have resulted in complete responses. On the "CxT" schedule, 6 of 14 courses have resulted in complete responses. A maximally tolerated dose has been defined for both schedules. The results of this study indicate that intrathecal AZQ has definite clinical activity in refractory meningeal malignancy, at a dose which is not associated with clinical toxicity.

3. Intrathecal 6-Mercaptopurine (6-MP) - 6-MP is an active antileukemic agent which has never previously been administered into the cerebrospinal fluid. Pre-clinical studies of intrathecal 6-MP, performed in a subhuman primate model indicated that 6-MP could be safely administered by the intrathecal route. Based on these studies we have initiated a Phase I study of intrathecal 6-MP in children with refractory meningeal malignancy. Both a twice weekly and a concentration x time (CxT) schedule (q12h x 6 doses) are being evaluated. To date, 6 of 9 patients with ALL treated on the twice weekly schedule have responded, 4 are complete responses. No significant toxicity has been observed. These results indicate that intrathecal 6-MP is safe and active against meningeal leukemia.

4. Thiotepea is an active alkylating agent with a steeper dose response curve than cyclophosphamide. This compound has been used with only marginal success via the intra-CSF route of administration. Our studies demonstrated, for the first time, that substantial amounts of both thiotepea and its metabolite Tepa are present in CSF following intravenous administration. This data indicates that this route of administration may be a more optimal one to approach CNS disease with this agent. As a result of these studies, Phase I study of intravenous thiotepea in pediatric patients has been developed and completed. Thiotepea was administered as an IV bolus on a q/3 week schedule; 65 mg/m<sup>2</sup> was identified as a safe dose for future Phase II trials. As a logical extension of this study we recently embarked on a multi-institutional Phase II study designed to assess the therapeutic efficacy of Thiotepea against brain tumors.

Thiotepea has a very steep dose response curve and its major toxicity is myelosuppression. In a recently instituted Phase I study, we are attempting to determine whether adjunctive administration of the hematopoietic growth factor GM-CSF will safely permit higher, potentially more effective, doses of systemic thiotepea to be administered.

5. Ongoing Phase I studies include Piritrexim and interleukin (IL-2). A Phase I trial of fludarabine phosphate was recently completed.

6. We have conducted a number of studies to evaluate the biochemical pharmacology, pharmacodynamics, and pharmacokinetics of those antileukemic agents used for maintenance treatment. Two examples of these studies include the following:

Chronopharmacology of Maintenance Therapy - A retrospective study recently cited five-fold greater risk of relapse in children with ALL who took their oral maintenance therapy on a morning rather than an evening schedule. A possible explanation was that the difference in the efficacy of the morning and evening schedules reflected a difference in total drug exposure that resulted from circadian periodicity in the disposition of 6-MP and MTX. We have investigated the chronopharmacokinetics of both 6-MP and MTX in children with ALL to determine if there is a pharmacokinetic basis for this

observation. Children were fasted and given either 6-MP or MTX at both 8:00 AM and 8:00 PM. No significant differences were noted in the plasma concentrations of either 6-MP or MTX on the two administration schedules. Thus, we are unable to confirm that diurnal variation in the absorption or elimination of 6-MP and MTX plays a role in the response to maintenance therapy with these drugs.

Subcutaneous MTX - We have studied subcutaneous MTX as a parenteral alternative to oral administration. The subcutaneous route has several potential advantages including slow release of the drug leading to more prolonged drug exposure, ease of administration, and more complete and less variable absorption. Two dose levels (7.5 and 40 mg/m<sup>2</sup>) were studied, and each child was monitored twice after an oral dose and after the same dose administered subcutaneously. The subcutaneous dose was well-tolerated and well-absorbed at both dose levels studied. In contrast, the oral dose produced comparable plasma concentrations at a lower dose, but total drug exposure (AUC) at the higher dose was only one-third that achieved with the subcutaneous dose, presumably a result of saturation of the mechanism responsible for MTX absorption in the gastrointestinal tract. Subcutaneous administration appears to be a viable alternative approach to oral maintenance therapy.

7. A detailed analysis of dose intensity (dose rate) in protocol 77-C-145 has been completed. A dose intensity of >80% for methotrexate and cyclophosphamide delivered during the first two cycles of therapy was shown to be significantly correlated with event-free survival. In a Cox multivariate analysis the tumor burden, as measured by the serum LDH level, and the dose intensity of methotrexate for cycle 2 were the most important determinants of event-free survival whether the analysis was carried out on the entire patient group, or in subgroups such as stages 1, 2 and 3 only, patients without bone marrow involvement, or in patients with small non-cleaved lymphomas. However, the dose intensity of drugs delivered in subsequent cycles was not of prognostic importance.

These findings strongly suggest that the chemotherapy dose rate is of critical importance early in therapy, at least for the small non-cleaved lymphomas which constitute the majority of patients. The rate of drug administration after cycle 3 appears to be unimportant, and it is probable that further chemotherapy after cycle 3 does not affect outcome. An additional finding was that patients with bone marrow involvement, as might have been anticipated, have a significantly lower dose intensity during the first two cycles of therapy. This is because bone marrow recovery is delayed in such patients. However, the correlation of dose intensity to outcome is not an exclusive result of the inclusion of patients with bone marrow involvement, since dose intensity remains important in multivariate analyses, and further, Cox models which do not include patients with bone marrow involvement also show dose intensity to be one of the two most significant prognostic variables. Moreover, apart from its inverse correlation to bone marrow involvement, dose intensity does not correlate with general measures of tumor burden such as clinical stage or serum LDH.

These findings suggest new strategies for patients with extensive disease, particularly in the presence of bone marrow involvement:

- a) Continuation of the second and third cycles of therapy regardless of the blood count, or
- b) Attempting to speed up bone marrow recovery, for example, by the use of colony stimulating factors.

The latter strategy is the one we have chosen to adopt.

8. A new protocol for the treatment of patients with non-lymphoblastic lymphomas has recently been approved by the clinical research subpanel, and is currently open for patient accrual. This protocol is based on observations made in previous Pediatric Branch protocols for the non-Hodgkin's lymphomas. Treatment consists of alternating cycles of regimens referred to as CODOX-M, a modification of protocol piloted as protocol 85-C67, and IVAC, a new regimen based on a pilot protocol, 85-C-62, which was used for the treatment of patients with recurrent non-lymphoblastic lymphomas.

The major goal of the new protocol is to determine whether GM-CSF administration will result in increased dose intensity (ie., dose rate) in high-risk patients, particularly those with bulky disease and/or bone marrow involvement, while at the same time decreasing toxicity. Patients will be randomized to receive either four cycles of CODOX-M/IVAC, or the same treatment accompanied by subcutaneously administered GM-CSF. In both therapy arms, sequential therapy cycles will be initiated as soon as the peripheral blood granulocyte count reaches 1000 per cu.mm. If GM-CSF treatment results in earlier bone marrow recovery, the interval between therapy cycles will be shortened and dose rate will therefore be correspondingly increased. The inclusion of a new regimen as well as GM-CSF in this protocol is justified since, apart from the uncertainty as to whether the strategy will be successful, control arm patients would otherwise be receiving a known ineffective protocol.

9. We continue to monitor results of the completed protocol which studied the intensive treatment program for patients with high-risk pediatric sarcomas. This protocol combined high-dose chemotherapy during induction with total body irradiation (800 rads) and autologous bone marrow reconstitution. Ninety-two percent of the patients enrolled on the protocol were successfully induced. The actual disease-free survival is 50% for those patients free of metastatic disease at diagnosis versus 20% for those with metastatic disease. These results are not significantly different from historical experience and therefore do not stimulate enthusiasm for further investigating a total body irradiation, autologous bone marrow transplant approach to the treatment of these diseases.

10. Protocol 87-C-10 a study of the treatment of moderate risk sarcomas with continuous infusion adriamycin as well as vincristine, cyclophosphamide, ifosfamide, and etoposide has been closed. The primary intent of the protocol was to determine whether continuous infusion of adriamycin would reduce cardiac toxicity. Of the seven patients treated on the protocol, there have been two cases of overt cardiomyopathy with one death. Two other patients have had a significant decrease in the muga scan ejection fraction. These results demonstrate that continuous infusion adriamycin is not likely to significantly reduce the cardiac toxicity associated with this agent. To further address this clinical problem protocol 89-C-07 has been initiated in tandem with the high-risk sarcoma protocol to determine whether the iron chelating agent ICRF-187 will inhibit adriamycin cardiotoxicity. Preliminary results from a study with

adults with breast cancer suggests that this is an active cardioprotective agent. Patients entered on protocol 86-C-169, the high-risk sarcoma protocol, will be randomized to receive ICRF-187 or not.

The pilot protocol for the treatment of high-risk sarcomas, 86-C-169, continues to accrue patients. There have been 43 patients entered. It is too early to judge the efficacy of the vincristine, cyclophosphamide, adriamycin, ifosfamide, and etoposide regimen. The major toxicity of the protocol, myelosuppression, is being addressed by a companion study, protocol 88-C-165, which is designed to determine whether the addition of the colony-stimulating factor GM-CSF will reduce the extent of myelosuppression in patients on the sarcoma protocol. Patients are being randomized to either receive or not receive the GM-CSF in conjunction with VAC and IE regimens.

11. To determine the role of new beta-lactam antibiotics in providing simpler, safer and effective therapy for neutropenic cancer patients who become febrile, we have conducted a randomized trial comparing a third-generation cephalosporin (ceftazidime) to a carbapenem (imipenem/cilastatin) for initial empirical therapy. The goal of this study is to both evaluate the role of these agents in providing safe initial therapy as well as determining whether the numbers of modifications of the primary antibiotic varies in patients with defined infection or prolonged granulocytopenia. From March, 1986-March, 1989, we enrolled 337 evaluation episodes of fever and neutropenia, randomizing these to initial ceftazidime (170 episodes) or imipenem (167). Both regimens provided comparable primary therapy. More modifications of the initial regimen were necessary for patients with documented infection who were randomized to ceftazidime and there were more second infections in this group. These were primarily with gram-positive bacteria. However, there were no differences in infection related morbidity or mortality. On the other hand, there were more complications with imipenem, including a higher incidence of C. difficile diarrhea and a higher degree of intolerance due to nausea and vomiting. Overall, both antibiotics appear useful, have different strengths and weaknesses and confirm that various alternatives can be employed to provide safe monotherapy for the majority of febrile neutropenic cancer patients.

12. We have continued our Phase I-II studies of children with symptomatic HIV infection. Since beginning this project in December, 1986, we have evaluated approximately 110 children, enrolling the majority into clinical trials.

Our initial study of AZT, administered either by continuous intravenous infusion or on an intermittent schedule, are completed. Both routes of therapy appeared to offer benefit, particularly for children with neurodevelopmental deficits. However, the extent of this benefit, appear to be greater for children treated by the continuous intravenous schedule. To validate this, we will soon begin a randomized study comparing AZT administered on a schedule that maintains steady-state kinetics in the plasma and CSF to one in which the drug is delivered on an intermittent schedule.

Our prior studies with AZT demonstrated that the dose-limiting toxicity was myelosuppression related to both dosage and duration. Consequently we have evaluated two schedules to spare AZT-induced myelosuppression. The first alternates AZT with ddC, and the second combines it with rH-GM-CSF. In the study with ddC, we first evaluated this newer dideoxynucleoside in a limited phase I trial, studying four dosage levels (0.015, 0.020, 0.030, and 0.040

mg/kg/q/6h) administered over an 8-week period. Fifteen patients were treated. We observed decreases in P24 antigen in 5/9, increments in CD4 counts in 8/15 during the 8-week trial of ddC as a single agent. We also treated 13 of these 15 patients with an alternating schedule of ddC and AZT and found this to be non-toxic and tolerable during a minimum follow-up period of 6 months.

We also initiated a protocol to evaluate the combination of AZT with colony stimulating factor in order to overcome the myelosuppression of AZT. To date, one patient has been treated on this protocol.

In a search for effective, less toxic regimen, we initiated a Phase I-II trial of dideoxyinosine (ddI) in children in January, 1989. To date, 21 children have been enrolled at several dosage levels (20, 40, 60, 120 mg/m<sup>2</sup>/ every 8 hours). This protocol enrolls both children who have received no prior anti-retroviral therapy as well as children who have become refractory or intolerant to AZT. To date, 21 children have been enrolled. The first 3 dose levels are completed in the 4th level (120 mg/m<sup>2</sup>/day) is accruing. No significant toxicity has been observed and both objective and subjective responses have been seen (albeit still preliminary) in both previously untreated and refractory/intolerant children.

### Pre-Clinical Studies

1. In the unique subhuman primate model for CSF pharmacokinetic studies, we have identified several approaches of potential utility in treating meningeal malignancy.

We have evaluated the feasibility of intrathecal administration of mafosfamide, a pre-activated analog of cyclophosphamide, which does not require hepatic activation, and thus is potentially feasible for regional administration. We have evaluated the pharmacokinetics of mafosfamide in the CSF following intraventricular administration. Our results indicate that therapeutic levels of this compound can be achieved in cerebrospinal fluid following intraventricular dosing. In addition, we have demonstrated that intrathecal therapy with this compound is feasible and safe. Further studies of this approach are being pursued in an effort to introduce this approach into clinical studies as rapidly as possible.

An innovative system which permits assessment of continuous intra-CSF drug administration has been developed in the subhuman primate. A unique lateral ventricular catheter attached to a continuous infusion portable pump, together with the 4th ventricular Ommaya reservoir enables us to deliver and monitor drug administration by continuous intra-CSF administration.

Using methotrexate as a model drug we have studied continuous intra-CSF administration and have demonstrated that 1) when compared to intraventricular bolus therapy continuous intraventricular infusion maintains target CSF methotrexate concentrations longer with a lower total dose, 2) continuous intra-CSF infusion avoids the high-peak methotrexate concentrations associated with bolus intrathecal dosing and thus may be less neurotoxic. Based on these studies a clinical trial evaluating this approach is being initiated.

2. We have studied the immunoglobulin and T-cell receptor gene configurations in acute lymphoblastic leukemia of childhood. The cells of 70 patients with ALL

have undergone immunophenotyping and molecular analysis. Objectives of the study were to investigate Ig and TCR genotypic patterns, and to determine any relationships between those patterns and clinical features at presentation in patients with this disease. A spectrum of genotypes was found, but with certain patterns predominating. Most (57/70) cases had rearrangements of both Ig and TCR loci, while 9 had rearranged Ig genes alone, and rearrangement of TCR but not Ig genes occurred in a single case. Rare leukemias (3/70) with all seven Ig and TCR genes germline were also identified. No significant relationships were found between molecular genotype and commonly used prognostic factors including age, sex, WBC, FAB subtype, and cytogenetics. The lymphoblasts of 3 of 6 patients who failed to achieve initial remission had germline patterns of all seven Ig and TCR loci, whereas the genotype was not found in any of 64 patients who had not failed induction ( $p=0.0007$ ). Thus, these studies indicate that at the molecular genetic level, B-cell precursor ALL of childhood is a heterogeneous disease. The observed association between induction failure and the genotype of all Ig and TCR loci germline warrants prospective evaluation to determine its clinical relevance.

3. In order to overcome drug resistance we must also define the mechanisms through which leukemic cells become resistant to the antileukemic agents. In collaboration with laboratories in the Medicine Branch, we have screened lymphoblasts from our patients on a molecular level for the presence of multidrug resistance caused by overexpression of mdr-1/P-170 and glutathione S-transferase (GST).

Lymphoblasts from 28 patients were studied for evidence of mdr-1/P-170, the gene encoding for the plasma membrane glycoprotein associated with multidrug resistance, using RNase protection, RNA in situ hybridization and immunohistochemistry. Overexpression with gene amplification was identified in the cells of three relapsed patients and from one patient at diagnosis (this patient failed to achieve a complete remission with induction therapy.) In situ hybridization, immunohistochemistry, and drug uptake studies demonstrate that this overexpression is heterogeneous. It appears from these studies that overexpression of mdr-1/P-170 is one mechanism of drug resistance in ALL.

GST is a drug metabolizing enzyme that is overexpressed in a multidrug resistance breast cancer cell line (MCF-7.) Expression of GST is being evaluated in lymphoblasts from our patients. Thus far, higher levels of expression have been found in the cells from two relapsed patients than in the cells from three patients obtained at diagnosis, suggesting that GST may also be a marker of acquired resistance in ALL. A technique to assess GST expression, which involves in situ hybridization, is currently being used to evaluate a large group of patients to determine the overall incidence of this phenomenon.

4. We have recently demonstrated that the polymerase chain reaction (PCR) can be used to identify chromosomal breakpoint locations in the small non-cleaved lymphomas. Making use of the repeat sequences in the u switch region, we have developed three different sets of oligonucleotide amplimers which are capable of amplifying fragments containing portions of both chromosomes 8 and 14 (ie. contain the breakpoint itself) and are also able to distinguish between three separate breakpoints in the regimen of the c-myc gene - breaks within the first c-myc intron, the first c-myc exon, or in the immediate c-myc 5' flanking sequences.

The clinical importance of these findings stems from the extremely high degree of sensitivity inherent in PCR. We have been able to detect, in artificially created cell mixtures, the presence of one cell in a million containing a breakpoint location in one of the regions that we are able to detect by PCR. Thus, this technique is not only capable of providing definitive diagnosis, but should enable us to identify very small numbers of cells in tissues not recognized microscopically as being involved. In addition, this technique can be used to follow the presence of minimal residual disease in the bone marrow after therapy, and could be of value in predicting which patients will relapse.

5. We have been able to specifically inhibit the expression of the c-myc gene in a subset of Burkitt's lymphomas. This has been accomplished by using an antisense oligonucleotide directed against intron sequences which are present in the mature messenger RNA species in Burkitt's lymphomas with c-myc first intron breakpoints on chromosome 8. Both cellular proliferation and c-myc protein expression were inhibited in the experiments. These findings demonstrate that the molecular abnormalities in tumors may also provide a target for specific therapeutic endeavors. Because only Burkitt's lymphoma cells, and not normal cells, contain the genetic abnormalities, such therapeutic approaches may be highly selective. We are pursuing pre-clinical studies with anti-sense oligonucleotides using Burkitt's lymphoma xenografts in a nude mouse model.

6. Utilizing a series of molecular markers that define specific stages during the maturation of human fetal adrenal neuroblasts, we have characterized a large series of human tumors to determine whether or not such tumors corresponding to stages in normal human development could be distinguished clinically. To date, our data suggests that the age of maturation to which the tumor cells can be recognized as corresponding is a strong indicator of outcome in both patients with limited stage and advanced stage neuroblastoma.

7. Utilizing in vitro assays to identify the maturation of human neuroblastoma tumor cells and cell lines, we have developed a line of investigation to identify molecular signals that mediate the maturation of this neuroendocrine lineage. The identification of such molecules should provide insights into both the cellular events critical for the regulation of maturation and the development of novel therapeutic agents.

8. We have extended our studies of insulin-like growth factor II in neuroblastoma and determined that tumors in which this gene is not expressed seem to have high levels of expression in a variety of cell types making up the stromal tumor compartment. The malignant cells of these same tumors also express high levels of the type I IGF receptor. We believe these results suggest that a paracrine growth mechanism may be of importance in mediating the growth of some neuroblastoma tumors.

9. We have cloned and sequenced a cDNA molecule encoding the glial fibrillary acidic protein, an intermediate filament whose expression is strictly limited to cells of the astrocytic lineage. In ongoing experiments we have isolated the genomic regulatory regions we believe critical for the regulation of GFAP expression and are currently pursuing the identification of cis-acting elements that mediate astrocyte specific gene regulation.



10. In our unique laboratory model of candidiasis we have developed methods in which to evaluate promising antifungal agents in various clinical settings. These include acute disseminated infection, chronic infection (e.g., hepatosplenic candidiasis), subacute or local infection and prophylaxis. This permits a more reliable assessment of antifungal strategies and has enabled us to determine that a new triazole, fluconazole, may offer benefit for early (e.g., prophylactic) use in neutropenic hosts. Accordingly, we are about to initiate a randomized clinical trial to assess the utility of prophylactic fluconazole in pediatric and adult cancer patients.

We have also studied the tissue and plasma pharmacokinetics of a number of other new antifungal agents, indwelling itraconazole, Sch 39304 and LY 121019. We have also tested all of these agents with fungal challenges and are assessing their role for clinical study.

We have also demonstrated important phenotypic alterations in Trichosporon beigelii, are emerging pathogens in cancer patients.

11. We have demonstrated that cytokines, including GM-CSF, overcome the defect in bacterial function that is present in pediatric AIDS patients and have begun to clarify the role of HIV-related glycoproteins in inhibiting phagocyte function.

#### RADIATION ONCOLOGY BRANCH

The Radiation Oncology Branch continues to meet its three major goals: 1) major emphasis on clinical trials of a combined modality nature, collaborative with other clinical branches; 2) a strong radiation biology program, with heavy emphasis on basic science, radiologic physics, and questions of clinical relevance; and 3) a training program in radiation oncology, equivalent to the stature of the programs of training in the NCI Medical, Surgical, and Pediatric Branches.

On-going clinical work focuses on small cell carcinoma of the lung, mycosis fungoides, soft tissue sarcomas, pediatric sarcomas, lymphomas, and Hodgkin's disease. These will be presented by other respective Branches, under whose aegis the protocols are carried out.

A primary ROB study centers on stage I and stage II breast cancer, in which patients with such carcinomas are randomized to receive either modified radical mastectomy or definitive radiation with preservation of the breast following a lumpectomy. With 240 randomized patients, despite the difficulties of such a randomization, there is no obvious superiority of either arm suggesting that long-term results of treatment will be comparable. This study differs from the studies that Fisher of the NSABP in that the surgical excision makes no attempt to have the margins surgically negative, but simply removes the gross lump. Cosmesis is a major end-point, in addition to survival and freedom from relapse. The studies are open to women who have masses up to 5 cm, with or without nodes, thereby making breast preservation applicable to the vast majority of women in this country who present with breast cancer. All patients with positive nodes receive adjuvant chemotherapy. The psychosocial aspects of these treatment approaches are being studied.

Halogenated pyrimidines have been investigated as radiosensitizers, especially IUDR. Our first phase I study of IUDR was completed with special attention to unresectable sarcomas and gliomas. Glioma information compares favorably to other reports, with a median survival of approximately 14 months in high-grade patients, and several patients live well beyond three years. In unresectable sarcomas, we've seen striking regressions of unresectable masses, and have several patients whose masses have gone away completely with IUDR and radiation of a phase I study. The probability of local control in unresectable sarcomas has been over 60% despite the fact that these masses are generally considered radioresistant and have been typically huge in size. We have just begun to take patients with unresectable sarcomas and randomized them to receive IUDR, "yes" or "no", in conjunction with radiation therapy. We've also begun a broad protocol for IUDR in a wide variety of cancers. We hope to investigate intra-arterial or intravenous IUDR, but this project has slowed because of the departure of a key neurosurgeon.

Photodynamic therapy has begun. We've utilized hematoporphyrin derivative and laser-controlled sources of light in the treatment of superficial cancers of the skin and mucous membranes. We've also used this for occluded bronchi and to peritoneal surfaces by means of intracavitary administration of the light. We hope to use the hematoporphyrin intracavitary as well, but have not yet been permitted by the FDA to do so. We also hope to use such treatment in the thorax. The preliminary results on the peritoneal cases with ovarian cancer appear quite promising, as part of a phase I study in patients with recurrent disease. We have escalated light doses at the time of abdominal exploration.

In the laboratory, the work is centered on the mechanisms of sensitization of protection, resulting from radiation modifiers, and the investigation of mechanism of action of several different cytotoxic agents. We have focused heavily on sulfhydryl compounds, especially glutathione, and its relationship to cell killing or protection by either radiation or chemotherapy. Additional work has gone on in heat shock proteins and in the characterization of human tumor cell lines in conjunction with other branches. The laboratory has demonstrated conclusively that cells, which are pleiotropically drug resistant, are not necessarily resistant to radiation therapy; moreover, such cells are definitely not resistant to photodynamic therapy. Under the direction of Dr. Gansow, we've been able to label a variety of monoclonal antibodies by means of newly-synthesized chelates to various isotopes. We have actually treated patients with radiolabeled antibody, but, at the moment, the only antibodies that have been studied in patients are B 72.3 labeled with iodine and T<sub>101</sub> labeled with yttrium. The patient accrual is modest, but toxicity has been mild so far. We hope to begin imaging studies with B-1 antibody against B cell lymphoma soon in preparation for treatment studies.

#### SURGERY BRANCH

Laboratory and clinical efforts of the Surgery Branch are concentrating on the development of new diagnostic and therapeutic techniques for the management of cancer patients.

#### Significant Laboratory Accomplishments

1. Experimental studies have shown that the adoptive transfer of tumor infiltrating lymphocytes plus IL-2 is from 50-100 times more effective than the

adoptive transfer of LAK cells in mediating the regression of established pulmonary and hepatic metastases in a variety of murine models. Recent experiments have shown that local radiation therapy provides synergistic effects with this adoptive immunotherapy.

2. Significant therapeutic synergies have been seen in animal models using combined lymphokine treatment. The most effective combined treatment includes the use of interleukin-2 and alpha-interferon. The combined administration of interleukin-2 and alpha-interferon also synergizes in this therapeutic effect with the adoptive transfer of tumor infiltrating lymphocytes.

3. Experimental techniques have been developed for inserting foreign genes into tumor infiltrating lymphocytes. Tumor infiltrating lymphocytes transduced with a gene for neomycin resistance can grow in high concentrations of the neomycin analog G418 that would be lethal to other cells. Initial studies following adoptive transfer in mice have begun. Work is in progress to transduce genes coding for lymphokines such as alpha-interferon and tumor necrosis factor into tumor infiltrating lymphocytes to increase their therapeutic effectiveness.

4. Tumor infiltrating lymphocytes have been isolated from patients with melanoma that have unique lytic specificity for the tumor from which they are derived and not for other normal tissues or other allogeneic tumors. The lysis by these cells is MHC restricted. Preliminary experiments have shown that specific lytic cells can be obtained from some patients with breast cancer and lung cancer.

5. In vivo administration of cytokines such as alpha or gamma interferon can result in upregulation of major histocompatibility antigens on murine tumors. This observation has formed the basis for combined therapy trials utilizing the interferons in conjunction with other cytokines in the adoptive transfer of MHC restricted killer cells.

6. In vivo treatment with cytokines such as tumor necrosis factor and gamma interferon can enhance the ability of cells to mediate antibody dependent cellular cytotoxicity. This has formed the basis for the development of new therapeutic protocols utilizing monoclonal antibodies and cytokine administration to treat established tumors.

7. Interleukin-4 has been found to synergize with interleukin-2 in the growth of melanoma specific tumor infiltrating lymphocytes. Further, it has been found that interleukin-4 inhibits LAK activity and regulates the responsiveness of lymphokine activated killer cell precursors to interleukin-2.

8. Pre-treatment of tumor cells with tumor necrosis factor and interferon gamma enhances the susceptibility to lysis by specific tumor infiltrating lymphocytes. This observation is being used in the design of clinical trials of combination immunotherapy.

9. Interleukin-6 has been found in the serum of mice and humans treated with tumor necrosis factor or interleukin-2 and alpha-interferon. Interleukin-2 treatment has been found to be associated with abnormal in vitro tests of neutrophil function, including altered migration and oxidative burst.

10. New techniques have been developed for extracting tumor infiltrating lymphocytes from murine tumors using immunomagnetic beads and from human tumors using plastic surfaces coated with anti cell-surface antibodies. These techniques have been used to raise TIL in mice with in vivo activity against non-immunogenic tumors.

11. We have demonstrated that advanced renal cell carcinoma is associated with DNA sequence deletions in the short arm of chromosome 11 and have performed deletion analysis to detect the area of shortest overlap. We have demonstrated that in some advanced renal cell carcinomas there are DNA sequence deletions at the retinoblastoma locus and that in some of the tumors the retinoblastoma gene is not expressed.

12. Molecular analysis of the short arm of chromosome 3 in renal oncocytoma, a non metastatic renal tumor, has shown that this non malignant renal tumor does not have DNA sequence deletions in the short arm of chromosome 3, therefore further supporting the hypothesis that abnormality at this locus is important for the initiation or progression of this renal cell carcinoma.

13. In studies of recessive oncogenes in human genito-urinary tumors we have previously described that there is a DNA sequence deletion in the short arm of chromosome 3 in human renal cell carcinoma. We have developed a new technique involving immunologic selection to remove contaminating leukocytes from primary tumors in order to significantly improve the detection of allele loss in human renal as well as other tumors.

14. Another genito-urinary tumor, adrenal carcinoma, is associated with DNA sequence deletions at chromosome 11 and chromosome 17.

15. To evaluate whether there is a diseased gene at the locus on the short arm of chromosome 3 in a familial form of renal cell carcinoma associated with von Hippel Lindau disease, we have demonstrated DNA sequence deletions on the short arm of chromosome 3 in not only renal cell carcinoma, but also in pheochromocytoma and spinal and cerebellar hemangioblastoma from patients with this disease. We have demonstrated that it is the wild-type allele (from the non-affected parent) which is deleted in the tumors and that the disease allele (originating from the affected parent) is retained in the tumor.

16. In studying the effect of suramin on human renal cell carcinoma, we have demonstrated that suramin partially reverses the growth inhibition induced by TGF-beta, that suramin has a direct, reversible inhibition of TGF-beta binding on renal cell carcinoma, and that suramin induces a dissociation of TGF-beta from its receptor. In a study of the effects of suramin on human prostate carcinoma, we have shown that suramin in various doses obtainable clinically, inhibits proliferation of prostate carcinoma in vivo in serum-containing as well as serum-free media. Studies are ongoing to characterize the mechanism of suramin on growth factor-mediated inhibition of prostate carcinoma growth and on the effect of the combination of agents on inhibition of prostate carcinoma growth.

17. Passive immunization with antibodies vs. murine TNF partially abrogates the lethality of IL-2 in mice. Antibody vs. TNF allows the administration of a significantly greater number of doses of IL-2 before the animal succumbs to IL-2

toxicity and dies. Antibody to TNF does not inhibit IL-2 induced anti-tumor efficacy.

18. Tolerance to TNF can be induced by repeated IP administration of TNF in animals, i.e. a lethal dose of TNF (a dose that would kill a naive animal) does not kill a tolerant animal. It also protects rats against endotoxin and a gram negative model of sepsis. Tolerance can be achieved by a single, low IV dose of TNF and may have relevance to the treatment of sepsis lethality.

19. Shown that murine macrophages can be activated by photodynamic therapy (PDT) to produce tumor necrosis factor.

20. Described the differential delivery of PDT sensitizer by the LDL pathway, and described inherent differences in lung cancer cell lines to in vitro PDT.

21. Reported the use of a photodiode system to quantitate light delivery for intra-abdominal and intra-thoracic PDT.

22. Defined the tolerance of native and prosthetic vascular grafts to intra-operative and conventional fractionated radiation therapy in canine model.

23. Defined tolerance of the peritoneal cavity and intra-abdominal viscera to photodynamic therapy using hematoporphyrin derivatives and laser light in a canine model.

24. Defined the tolerance of gastrointestinal anastomoses and suture lines to intraperitoneal photodynamic therapy.

25. Interleukin-2 inhibits hormone-dependent breast cancer cells in vitro in a dose dependent manner; this inhibitory action is not associated with alteration in gene expression of growth factors TGF alpha, TGF beta, or EGF receptor.

26. Estrogen receptors of human breast cancer cells exist in discrete large molecular weight forms which are not dissociated by high salt and require protolytic digestion for conversion to the subunit form.

27. Interleukin-1 down-regulates estrogen receptor activity in a time-dependent and dose-dependent manner without altering affinity for estradiol in MCF-7 cells. Interleukin-1 antagonizes estradiol (E<sub>2</sub>) stimulation of growth in vitro and E<sub>2</sub> stimulation of progesterone receptor (PR) synthesis without altering the PR dissociation constant in MCF-7 cells.

### Significant Clinical Accomplishments

1. Clinical trials with lymphokine activated killer cells and interleukin-2 or the administration of high-dose interleukin-2 alone have demonstrated that approximately 10% of patients with metastatic melanoma or metastatic renal cell cancer will undergo a complete regression of all cancer and approximately 1/3 of patients will undergo at a 50% regression of malignancy.

2. A prospective randomized trial has been completed comparing the use of lymphokine activated killer cells and IL-2 with interleukin-2 alone in the treatment of patients with advanced cancer. These studies have shown that an increased incidence of complete regression is seen in patients treated with LAK

cells and IL-2. No difference in overall regression rates (PR + CR) were seen. A clinical trial has been completed in 20 patients with advanced malignant melanoma treated with tumor infiltrating lymphocytes plus IL-2 that has demonstrated a 50-60% response rate in these patients.

3. Clinical protocols have been performed using a combination of alpha-interferon and IL-2 in escalating doses which suggests that the combination of these two cytokines provides higher response rates in advanced cancer patients than the administration of each cytokine administered alone.

4. Phase I clinical trials using GM-colony stimulating factor had been completed and the dose limiting toxicities have been defined. Clinical trials utilizing GM-CSF in combination with cyclophosphamide demonstrated that the period of neutropenia can be decreased using GM-CSF.

5. Prospective randomized clinical trials of patients with soft tissue sarcomas continue to reveal an advantage to adjuvant chemotherapy in patients following surgical resection of their primary sarcoma. Current trials are evaluating the use of radiation therapy in the treatment of these patients.

6. Combinations of interleukin-2 with murine monoclonal antibodies to melanoma and colorectal carcinoma have been carried out. In the presence of radiolabeled antibodies, IL-2 has been demonstrated to prolong the whole body retention of antibody and to increase the apparent delivery to tissues. Clinical trials using combinations of IL-2, monoclonal antibodies at high doses and LAK cells are now in progress.

7. Prospective randomized trials of intraoperative radiation therapy are in progress in the treatment of gastric cancer. Prospective randomized trials of patients with pancreatic cancer and retroperitoneal sarcomas have been concluded. At the present time, improved local control has been demonstrated, but no improvement in disease-free or overall survival has been seen in patients receiving intraoperative radiation therapy compared to patients receiving conventional external beam radiation therapy.

8. Clinical trials using escalating doses of IL-4 have been initiated in patients with advanced cancer.

9. Combinations of high-doses of monoclonal antibodies and IL-2 can be administered safely to patients with cancer and may be associated with decreased development of human anti-mouse antibodies.

10. A prospective randomized trial for stage I, II breast cancer comparing modified radical mastectomy with lumpectomy/axillary dissection/breast radiotherapy, at median followup of 5-1/2 years, shows no difference in overall or disease-free survival between the two treatment groups. The breast recurrence rate is approximately 19% and does not correlate with tumor size, nodal or receptor status, or intraductal component.

11. Intensive preoperative chemotherapy (CAMFPT) does not increase the hospital or operative course or postoperative complication rate for mastectomy for locally advanced stage IIIA,B breast cancer.

12. Developed techniques for delivery of photodynamic therapy and treated 15 patients with disseminated intraperitoneal malignancies with intra-abdominal photodynamic therapy in Phase I study.

13. Reported the treatment of patients with bronchial obstruction by photodynamic therapy at comparable dose rates and energy.

14. Fifty percent of patients with MEN-1 and ZES, who have an islet cell tumor imaged on preoperative CT or angiogram, have a malignant islet cell carcinoma on exploration based on pathological evidence of a primary tumor with metastases. Patients with MEN-1 and ZES are not cured of the biochemical manifestations of ZES by islet cell tumor resection, but similar patients with insulinoma or VIPoma can be cured.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 07208-01 CO

## PERIOD COVERED

October 1, 1988, through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anti-HIV activity of phosphorothioate analogues of oligodeoxynucleotides

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Robert Yarchoan, M.D., Senior Investigator, Clinical Oncology Program, NCI  
Makoto Matsukura, M.D., Expert, Clinical Oncology Program, NCI  
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## COOPERATING UNITS (if any)

Applied Biosystems, Inc.: Dr. Gerald Zon

## LAB/BRANCH

Clinical Oncology Program, Office of the Associate Director

## SECTION

## INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

## TOTAL MAN-YEARS

## PROFESSIONAL:

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Nuclease-resistant phosphorothioate analogues of oligodeoxynucleotides were synthesized by sulfurization of either internucleoside phosphite linkages, in a repetitive manner during chain extension, or internucleoside hydrogen phosphonate linkages, in a single step following chain assembly. These analogues were tested as antiviral agents against human immunodeficiency virus (HIV). In a cytopathic effect inhibition assay using HIV-uninfected susceptible T-cells (tetanus-toxic specific normal T-cells) co-cultured with irradiated, chronically HIV-infected cells, phosphorothioate oligomers inhibited the cytopathic effect and replication of several isolates of HIV-1 and HIV-2. Thus phosphorothioate analogues of oligodeoxynucleotides could inhibit cell-to-cell transmission of the virus as well as the infection by cell-free virus particles and also could inhibit variety of isolates of human retroviruses.



## INTRODUCTION

Antisense oligos, which are complementary to viral genome sequences, are reported to have inhibitory effects on Rous sarcoma virus (Zamecnik, et al., 1978), human immunodeficiency virus (HIV) (Zamecnik, et al., 1986), vesicular stomatitis virus (Agris, et al., 1986; Lemaitre et al., 1987), herpes simplex virus (Smith, et al., 1986) and influenza virus (Zerial et al., 1987). However, the susceptibility of unmodified, "normal" oligos to degradation by nucleases could make them less potent or less persistent against viruses. Phosphorothioate analogues of oligos, in which one of the non-bridging oxygen atoms in each internucleotide phosphate linkage is replaced by a sulfur atom, have several properties that make them potentially advantageous analogues: (a) resistance to cleavage by nucleases; (b) properties similar to normal oligos and higher  $T_m$  (stronger hybridization) (Zon, 1988; Stein et al., 1988) than methylphosphonate analogues, which are also nuclease resistant (Smith et al., 1986). These factors led us to test the phosphorothioate analogues as antiviral compounds against HIV.

## EXPERIMENT AND DISCUSSION

A. Preparation of phosphorothioate analogues of oligodeoxynucleotides

All of the compounds were prepared by either stepwise or single-step sulfurization methods implemented on an Applied Biosystems Model 380B DNA Synthesizer that employed manufacture-supplied reagents and solvents, except for the sulfurization step and the pre(post)-sulfurization wash steps, which used freshly prepared solutions B and A, respectively: solution B= 2.5 g sulfur (Aldrich, >99.999%), 23.7 ml carbon disulfide (Aldrich, HPLC grade), 23.7 ml pyridine (Aldrich, highest purity, anhydrous) and 2.5 ml triethylamine (Aldrich, highest purity): solution A= 50 ml carbon disulfide and 50 ml pyridine.

1. Stepwise sulfurization The following method is an improved version of the procedure reported by Stec et al. (1984). *O*-Methyl phosphoramidites were used with the following modifications to manufacture-supplied cycles for "tritylon" synthesis on either the 1- $\mu$ mol or 10- $\mu$ mol scale. Oxidation with iodine-water lutidine was replaced with sequential delivery of acetonitrile, solution A, solution B (then wait 450 sec), solution A, and acetonitrile; detritylation with trichloroacetic acid employed 100- and 180-sec delivery times for 1- $\mu$ mol and 10- $\mu$ mol scales, respectively. The deprotection cycle with thiophenol was unmodified.

2. Single-step sulfurization Hydrogen-phosphonate monomers were used with manufacturer-supplied cycles for "trityl-on" synthesis with either the 1- $\mu$ mol or 10- $\mu$ mol scale employing a new, stable activator (1-adamantanecarbonyl chloride) and an essential capping reagent (isopropyl phosphite) (Andrus, et al., 1988). Manual sulfurization with solution B was carried out according to manufacturer specifications (Applied Biosystems Model 381A User Bulletin 12, December 1987). The ammonia from the deprotection process (55°C, ~15 h) was removed in the presence of triethylamine (to preserve the trityl group) in the usual manner (Applied Biosystems Model 380/381 User Bulletin 13 (Revised), April 1987), and the resultant crude product was purified with either an oligo purification cartridge (McBride, et al., 1988) (OPC<sup>TM</sup> method, Applied Biosystems) or by HPLC using a reversed-phase (C<sub>8</sub> or

C<sub>18</sub> or polystyrene) column and a linear gradient of increasing acetonitrile concentration (20-50%) over 30 min, in 0.1 M triethylammonium acetate, pH 7.0 (Stec, et al., 1985). The detritylated, purified product was freed of residual triethylammonium acetate and obtained as the sodium salt by ethanol-precipitation from aqueous NaCl (ca. 70-80% v/v ethanol and ca. 0.3-0.15 M NaCl).

**B. Cytopathic effect inhibition assay** We previously reported this assay method (Mitsuya, et al., 1984 and Matsukura, et al., 1987). For this assay, we used two cell lines (ATH8 cells and TT12 cells) as target cells because they are profoundly sensitive to the cytopathic effect of HIV. In this paper, we report the method and results of assays using tetanus-toxoid specific normal helper/inducer T-cells (TT12). TT12 cells are one of clones generated in our laboratory (Mitsuya, et al., 1987). TT12 cells were stimulated by soluble tetanus-toxoid antigen plus irradiated (4000 rad) autologous accessory cells 4 days before the experiment. TT12 cells ( $2 \times 10^5$ ) were pretreated with 2  $\mu$ g/ml of polybrene (Sigma) for 30 min, pelleted and suspended in 2 ml of IL-2 containing (15% conventional IL-2: Advanced Biotechnology, Inc. plus 20 units/ml recombinant IL-2: Amgen Biologicals) complete media co-cultured with  $4 \times 10^5$  lethally irradiated (10,000 rad) HIV-producing cells (H9/ HTLV-III<sub>B</sub>) in the presence or absence of oligomers.

Number of viable cells were counted on day 10 after the exposure to virus in a hemocytometer under the microscope by the trypan blue dye exclusion method. We used S-dC<sub>28</sub> for the assessment of antiviral activity because S-dC<sub>28</sub> was found to be one of most potent sequences in the assay using ATH8 cells (Matsukura, et al., 1987). Normal helper T-cells were protected by S-dC<sub>28</sub> against cell-to-cell transmission and cytopathic effect of HIV-1 without toxicity of oligo at the concentrations from 0.5  $\mu$ M to 10  $\mu$ M (Fig.2), which is similar to the previous observations in ATH8 assay system (Matsukura, et al., 1987).

In a similar assay system using H9/RFII, the one of most divergent variant of HIV-1 (Hahn et al., 1985), and normal helper T-cells (TT12) were protected by S-dC<sub>28</sub>. It was also found that cytopathic effects of HIV-2 (Clavel, et al., 1986) were inhibited by S-dC<sub>28</sub> at 0.3  $\mu$ M (unpublished result). Furthermore, phosphorothioate oligos showed antiviral activity against caprine arthritis encephalitis virus (CAEV) (unpublished data). Thus phosphorothioate oligomers showed antiviral activity against de novo infection of various retroviruses, which does not require ordered sequences such as antisense configuration (sequence non-specific antiviral activity). However, as we initially expected, there appears to be sequence-specific antiviral activity in another assay system using chronically infected cells to study inhibitory activities of oligos to viral expression of HIV. Preliminary results in this assay system showed that 28-mer antisense phosphorothioate oligo against art/trs sequence of HIV-1 could specifically inhibit the viral protein production, but homo-oligos such as S-dC<sub>28</sub> failed to inhibit (unpublished). Therefore, there could be at least two different mechanisms, namely inhibition of de novo infection and inhibition of viral expression of HIV. The latter mechanism apparently requires higher concentrations of phosphorothioate oligos and a specific sequence, namely "antisense". In addition, there seems to be several other properties of phosphorothioate oligos such as inhibitory activity against syncytia formation induced by HIV and protective effect for macrophage against HIV-induced dysfunction (unpublished results). Determination of the

precise mechanisms requires further research. Nevertheless, phosphorothioate analogues of oligodeoxynucleotides might represent new agents against HIV and related viruses.

#### PUBLICATIONS

1. Matsukura M, Zon G, Shinozuka K, Stein CA, Mitsuya H, Cohen JS, Broder S. Synthesis of phosphorothioate analogues of oligodeoxyribonucleotides and their antiviral activity against human immunodeficiency virus (HIV). *Gene* 1988;72: 343-7.
2. Matsukura M, Zon G, Shinozuka K, Robert-Guroff M, Stein CA, Mitsuya H, Wong-Staal F, Cohen JS, Broder S. Regulation of viral expression of HIVB (human immunodeficiency virus) in vitro by antisense phosphorothioate oligodeoxynucleotide against rev (art/trs). *Proc Natl Acad Sci USA* 1989;86:4244-8.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 07209-01 CO

## PERIOD COVERED

October 1, 1988, through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Administration of 2',3'-dideoxyinosine (ddi) for severe HIV infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

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## COOPERATING UNITS (if any)

DCT, DTP: Dr. Neil Hartman, Dr. David G. Johns

Abbott Laboratories: Dr. Jean-Pierre Allain

## LAB/BRANCH

Clinical Oncology Program, Office of the Associate Director

## SECTION

## INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

## TOTAL MAN-YEARS

## PROFESSIONAL:

## OTHER

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Seven dose regimens of 2',3'-dideoxyinosine (ddI), a nucleoside analog never previously given to humans, were administered to 23 patients with acquired immunodeficiency syndrome (AIDS) or severe AIDS-related complex (ARC). Nine of these patients had previously been intolerant of AZT. Patients received ddI intravenously, either 2 or 3 times daily for 2 weeks, and then received the drug orally for 4 to 28 weeks at twice the intravenous dose. ddI was well absorbed from the gut when administered with antacids and it penetrated into the cerebrospinal fluid. Little or no evidence of a clinical anti-HIV effect was seen at the lowest two doses. However, the patients in the 5 highest dose groups (0.8mg/kg ddI intravenously and then 1.6 mg/kg orally every 12 hrs or higher doses) had increases in their absolute number of T4 cells, their total lymphocytes, and their T4/T8 ratio by week 6. Four previously anergic patients developed delayed type hypersensitivity skin test reactions during treatment. Each of the 6 patients in the 5 highest dose groups who had detectable serum HIV p24 antigen at entry had decreased p24 antigenemia by week 6. Finally, 12 of the patients reported increased appetite, and overall the patients gained 1.3 kg weight during the first six weeks of therapy. Adverse effects which may possibly have been related to ddI administration included seizures in 2 patients, a rash in 1 patient, neutropenia in 1 patient (who was also receiving trimethoprim/sulfamethoxazole), and mild irritability headaches or insomnia in approximately half the patients. Neither anemia nor peripheral neuropathy attributable to ddI administration was observed. These results suggest that clinical activity of ddI against HIV can be observed at doses which are tolerated without major toxicity in the majority of patients in short term regimens.

## INTRODUCTION

The development of 3'-azido-2',3'-dideoxythymidine (zidovudine, AZT), a dideoxypyrimidine, demonstrated that antiretroviral therapy can reduce the morbidity and mortality of patients with severe HIV infection. However, the use of AZT is limited by the development of substantial toxicity in a high percentage of AIDS patients. This coupled with the identification of AZT-insensitive viral isolates in some patients on long-term therapy makes the development of other agents an urgent priority. 2',3'-dideoxyinosine (ddI) is a purine dideoxynucleoside with potent activity against HIV *in vitro* in T cells and monocytes at concentrations of 10  $\mu$ M. Upon entering human cells, ddI can be converted through a complex series of reactions to its active moiety, 2',3'-dideoxyadenosine-5'-triphosphate (ddA-TP). In this form, it is thought to inhibit HIV DNA polymerase (reverse transcriptase) activity and thereby suppress HIV infection by blocking the synthesis of a DNA copy of the viral genome. Its mechanism of action is thought to be competitive inhibition of reverse transcriptase, chain-termination, or both.

ddI was found by Mitsuya and Broder to have a relatively high therapeutic index *in vitro*, as compared to other dideoxynucleosides when tested against HIV in human T cells. In addition, *in vitro* studies demonstrated that ddI had relatively little toxicity for human bone marrow precursor cells. Finally, studies in dogs showed that levels of 100  $\mu$ M of ddI caused little short-term toxicity. These observations suggested that ddI was worth testing clinically in patients with HIV infection. In this project, we report the first clinical study of this agent designed to test the feasibility of administering it to patients with AIDS or AIDS-related complex (ARC) and its activity against HIV.

## PATIENTS AND METHODS

### PATIENTS

23 patients (22 male and 1 female) with HIV infection, aged 23 to 51, were entered into this initial clinical trial of ddI. 21 of the patients were gay men, 1 had a history of intravenous drug use, and 1 had had heterosexual contact with an infected individual. Five of the patients had AIDS with a history of *Pneumocystis carinii* pneumonia, 1 had AIDS with a history of esophageal candidiasis, 3 had AIDS with Kaposi's sarcoma, and 14 had ARC. All the ARC patients had either a history of oral candidiasis or had recently lost 10% or more of their body weight. All the patients had antibodies to HIV and had a helper T cell ( $CD4^+$  cell) count below 300 cells/ $mm^3$  (median 60; range 6-266).

In selecting patients for this study, preference was given to patients who had not received AZT or had received it for 4 months or less because of concerns that patients who had had long-term AZT therapy might not be able to manifest immunologic improvement in response to another dideoxynucleoside. Eleven patients had previously received AZT, 1 for two years and the other, 10 for 2 weeks to 4 months. Nine of these 11 patients were intolerant of AZT by virtue of having developed nausea, malaise, headaches, or fevers while on this medication; the other 2 patients had stopped AZT to enter this study. All but 1 patient (no. 6) did not receive AZT or experimental anti-HIV therapy during the 4 week preceding their entry into the study.

### Drug Regimen and Monitoring of Patients

Each patient received an intravenous test dose of ddI over 1 1/2 hrs, followed by an oral test dose the following day. The patients were then given ddI intravenously for 14 days according to the following regimens: regimen A, 0.2 mg/kg every 12 hr; regimen B, 0.4 mg/kg every 12 hr; regimen C, 0.8 mg/kg every 12 hr; regimen D, 0.8 mg/kg every 8 hr; regimen E, 1.6 mg/kg every 12 hr; regimen F, 1.6 mg/kg every 8 hr; regimen G, 3.2 mg/kg every 12 hr. Each dose was administered over 1 1/2 hrs. Patients who completed the intravenous therapy then received ddI orally at twice the intravenous dose at the same dosing schedule for an additional 4 weeks. Because ddI is acid labile, oral doses were administered in apple juice 2 minutes after the patient ingested 30 cc of an antacid (either magnesium hydroxide and aluminum hydroxide suspension or aluminum hydroxide suspension). Patients who completed this initial 6-week period of dosing were given the option of continuing on oral ddI. As experience was gained with higher doses, patients initially started on lower doses were escalated to a maximum of 1.6 mg/kg ddI orally every 8 hrs. Patients who started receiving oral therapy at higher doses than that were maintained on these respective higher doses.

Patients were closely observed for toxicity and monitored for clinical and laboratory changes. They were tested for cutaneous delayed-type hypersensitivity at entry and at the end of 6 weeks of therapy with 0.1 ml candida extract, intermediate strength (5 TU) purified protein derivative (PPD), tetanus toxoid, and trichophyton extract as previously described. Tests were considered positive if 10 mm or more induration was observed at 24 or 48 hrs. Lymphocyte subsets reacting to Leu 3 (T4<sup>+</sup>, helper-inducer T cells) or to Leu 2 (T8<sup>+</sup>, suppressor-cytotoxic T cells) were analyzed by flow cytometry; monocytes were gated out in this analysis. Absolute values of these parameters were calculated as the total white blood count multiplied by the percentage of lymphocytes multiplied by the percentage of lymphocytes reacting with the monoclonal antibodies by flow cytometry. The percentage of lymphocytes was determined by an automated counter in which 10,000 leukocytes are analyzed. In patients, where two pre-treatment analyses of lymphocyte subsets were attained in the month prior to entry, the mean of these values was used as the baseline value. Patients were also assessed for their ability to mount an *in vitro* proliferative response to tetanus, diphtheria, and A/Mississippi/85 influenza virus as previously described. Serum obtained at entry, week 2, and week 6 was assayed for HIV p24 antigen using an enzyme-linked immunosorbent assay (ELISA) developed by Abbott Laboratories.

On certain days, heparinized plasma samples were obtained at various times after dosing for measurement of ddI. In 2 patients, simultaneous cerebrospinal fluid (CSF) and plasma samples were obtained 1 hr after the completion of an intravenous dose. ddI concentrations in the plasma and CSF samples were measured by high-performance liquid chromatography (N.R. Hartman, J.A. Kelly, and D.G. Johns, unpublished).

## Statistics

The changes in mean lymphocyte counts, T cell subsets, and HIV p24 antigen were assessed by the two-sided Wilcoxon signed-rank test for paired observations,  $p < 0.05$  being regarded as significant. The T4/T8 ratio was logarithmically transformed prior to analysis. For purposes of statistical calculations, all patients who completed 2 or more weeks of therapy are considered evaluable.

## RESULTS

### Clinical Pharmacology

The peak ddI concentration was roughly proportional to the administered dose and ranged from 0.5  $\mu\text{M}$  in patients receiving 0.2 mg/kg intravenously to 10  $\mu\text{M}$  in patients receiving 3.2 mg/kg intravenously. The average plasma half-life was about 35 minutes. The oral bioavailability of liquid ddI administered 2 minutes after antacids averaged 35%. Finally, the CSF/plasma ratio averaged 0.19 1 hr after the completion of an intravenous infusion of ddI. Thus, ddI could be administered orally and at least partially penetrated into the CSF.

### Clinical Evaluation

Three patients (nos. 5, 18, and 22) did not complete the first 6 weeks of dosing as planned. One of these patients (no. 5) was diagnosed as having Cryptococcal meningitis at day 8, one patient (no. 18) elected to leave the hospital after one week to attend to an ill family member, and one patient (no. 22) on dose group G developed a seizure after 5 weeks of ddI therapy. All of the other patients completed at least 6 weeks of therapy as planned, and patient 18 was started again on oral ddI. All of the patients who completed 6 weeks elected to continue on oral ddI beyond this period, and all but 6 of the 23 patients entered are presently receiving ddI 6 to 29 weeks after their entry into the study. No patient has expired.

During the first 6 weeks, one patient (no. 13) developed a morbilliform pruritic rash which subsequently subsided even though he was continued on ddI. Thirteen of the patients complained of mild headaches, restlessness, or insomnia. These symptoms generally did not interfere with work, were most pronounced during the second to fourth weeks of therapy, and then subsided somewhat. They occurred most frequently in patients who had previously been intolerant of AZT because of constitutional symptoms. Several patients complained about mild diarrhea which was attributable to the magnesium hydroxide and aluminum hydroxide suspension and which resolved upon using aluminum hydroxide as the antacid. Finally, as noted above, one patient (no. 22) developed a seizure on week 5; an evaluation of this patient showed no mass lesion although an electroencephalogram performed one week after ddI had been discontinued showed a focal abnormality. We did not observe peripheral neuropathy as a side-effect. Bone marrow toxicity was not observed during the initial 6 weeks, and in contrast to the macrocytic changes observed with AZT administration, there was no overall increase in the red blood cell mean corpuscular volume (MCV). Finally, the patients on the highest dose of ddI averaged a 1 to 2 mg/dl increase in their serum uric acid, most likely as a result of ddI catabolism, but this was not clinically significant. Thus, except for the patient who seized (and we do not know if this was a side effect

of ddI), toxicity was minimal during this initial therapy, even in the 9 patients who had previously been intolerant of AZT.

For the most part, ddI was well tolerated during extended dosing. In total, 4 patients were taken off ddI drug after the initial 6 weeks of therapy: 2 because of seizures (nos. 4 and 7); 1 because of neutropenia (no. 1); and 1 at the patient's request because of the inconvenience of participating in the protocol (no. 2). Of the 2 patients who developed seizures, one (no. 7) was found to have cerebral toxoplasmosis while a preliminary evaluation of the other patient has not revealed any underlying structural lesion. Both were receiving 1.6 mg/kg ddI every 8 hours. The patient who became neutropenic ( $<500$  neutrophils/mm<sup>3</sup>) was receiving trimethoprim/sulfamethoxazole prophylaxis for Pneumocystis carinii pneumonia. Finally, two patients (nos. 1 and 12) developed Pneumocystis carinii pneumonia, one patient (no. 12) developed bacterial pneumonia, and one patient (no. 3) complained of increased forgetfulness.

Twelve of the patients reported increased energy, reduced fatigue, or decreased sleep requirements upon being started on ddI. The one patient (no. 6) with arthralgias at entry reported improvement in this symptom. Finally, 4 patients reported an increase in their appetite, and overall, the patients on this study gained an average of 1.3 kg weight during the first 6 weeks of ddI. This weight gain was not associated with clinical evidence of fluid retention.

#### Immunologic and Virologic Changes

Overall, there was no clear trend in the T4 cells in the patients on the lowest dose group (dose group A), and in dose group B, there appeared to be only a transient increase during the first two weeks of therapy. In the 5 subsequent groups, however, sustained increases in T4 cells were observed during the 6 weeks of therapy. Each of the 15 evaluable patients in dose groups C through G had an increase in their T4 cells at week 2 ( $p<0.001$ ), and in all but one of these patients, the increase was sustained at week 6 ( $p<0.002$  compared with entry). For these 15 patients, the mean number of T4 cells increased from  $89 \times \pm 1.35/\text{mm}^3$  at entry (geometric mean  $\times \pm \text{SEM}$ ) to  $131 \times \pm 1.31$  at week 6.

Along with the increase in T4<sup>+</sup> lymphocytes, the patients on the 5 highest dose groups also had an increase in their T4/T8 ratios and the total lymphocyte populations. Again taking the patients in dose groups C through G, the mean T4/T8 ratio increased from  $0.16 \times \pm 1.27$  at entry to  $0.23 \times \pm 1.25$  at week 2 ( $p<0.01$ ) and  $0.22 \times \pm 1.28$  at week 6 ( $p<0.05$  compared to entry). As for the absolute lymphocyte count in the patients in dose groups C to G, this increased from  $1107 \times \pm 1.09$  at entry to  $1411 \times \pm 1.09$  at week 2 ( $p<0.01$ ) and  $1316 \times \pm 1.08$  at week 6 ( $p<0.01$  compared with entry). There was a slight increase in the T8 cells in the patients during therapy (from  $451 \times \pm 1.16$  cells/mm<sup>3</sup> in the patients in dose groups C to G at entry to  $562 \times \pm 1.14$  cells/mm<sup>3</sup> at week 6), but this was not statistically significant ( $p>0.05$ ).

Fifteen of the patients failed to mount a delayed-type cutaneous hypersensitivity reaction to any of the four test antigens at entry. Of these 15 anergic patients, four (pts. 9, 10, 17, and 21) developed a hypersensitivity response (i.e. developed 10 or more mm of induration at 24 or 48 hrs) to at least one antigen when retested after 6 to 8 weeks of ddI. Also, none of the



patients who had a positive skin test at entry lost this response when retested at 6 weeks. In 13 of the patients, T cell proliferative responses to a mitogen (pokeweed mitogen) and soluble antigens (tetanus and diphtheria toxoids) were measured at entry and after 6 weeks of therapy. Of these patients, three had a 3X or greater increase in the response to pokeweed mitogen, four had a 3X or greater increase in the response to tetanus toxoid, and one had a 3X or greater increase in the response to diphtheria. None of the patients who had increased proliferative responses were in the lowest two dose groups. Thus, some of the patients in dose groups C to G showed evidence of having an improvement in their T cell function in conjunction with an increase in the absolute number of T4 cells.

Of the 21 evaluable patients, 9 had detectable serum HIV p24 antigen at entry. Three of these 9 patients were in the lower 2 dosing groups; these 3 patients did not appear to have a decrease in p24 antigen upon administration of ddI. However, all 6 antigenemic patients who were in dose groups C to G had a decrease in their p24 antigen at weeks 2 and 6 ( $p < 0.05$  compared to entry). In addition, the three antigenemic patients who were entered in dose groups F or G all became p24 antigen negative (i.e.  $< 21$  pg/ml) by week 6 of therapy. In some patients who received 1.6 mg/kg ddI orally every 8 hrs, there was a late increase in serum p24 antigen after the initial 6 weeks of therapy. However, in all the evaluable patients who received 3.2 mg/kg of ddI orally every 8 hr or higher doses, the serum p24 antigen remained low or undetectable during the extended therapy. Finally, none of the patients who were serum p24 antigen negative at entry developed detectable antigen during their therapy with ddI. These results suggest that regimens of 3.2 mg/kg ddI every 8 hrs or higher dosing schedules may give a more sustained virustatic effect. Additional studies, however, will be needed to assess this point.

## DISCUSSION

ddI is a dideoxypurine nucleoside analogue. It is closely related to dideoxyadenosine, a compound first synthesized by Robins and Robins in 1964. The results of this pilot study demonstrate that ddI can be absorbed orally when given with antacids and that it penetrates the cerebrospinal fluid. In addition, they show that ddI can be administered to HIV-infected patients for 6 months with minimal toxicity. Finally, the results show that higher doses of ddI can induce an increase in the number of circulating T4 cells and total lymphocytes, a fall in serum HIV p24 antigen, and in some cases improvement in T cell function when administered to patients with AIDS or severe ARC. It is noteworthy that these changes occurred even though the drug was given at intervals of 8 to 12 hours.

At present, AZT, a dideoxypyrimidine, is the only antiretroviral drug which has been formally shown to prolong the life of patients with AIDS or related disorders. However, 40 to 80% of AIDS patients, especially those with advanced disease, may not be able to tolerate AZT for 6 months because of bone marrow suppression, nausea, or other toxicities. Also, the clinical and immunologic improvement induced by AZT is only transient in many patients with AIDS; after 24 weeks, the number of T4 cells in patients receiving AZT is not different than patients on placebo, and some patients on a constant dose of AZT have late rises in their serum HIV p24 antigen. Finally, Larder et al. have recently reported

that HIV isolates from patients receiving long-term AZT therapy may become less sensitive to this drug although the clinical significance of this laboratory observation is not known. A related dideoxypyrimidine, 2',3'-dideoxycytidine (ddC), was also shown to reduce HIV viremia and induce immunologic improvement in Phase I studies. The continuous administration of high doses of ddC, however, is frequently associated with the development of peripheral neuropathy. Regimens involving lower doses of ddC are currently being explored, and preliminary results suggest that an anti-HIV response can be attained with a dosing regimen that does not cause peripheral neuropathy in most patients. Also, preliminary results of a study of AZT and ddC suggest that the toxicities of these two drugs can be reduced by administering them in an alternating combination regimen, each drug being given for a week at a time. However, while the toxicities of these two drugs can partially be addressed in this manner, additional therapeutic agents are clearly needed.

ddI, a dideoxypurine, was found in our laboratory to have activity against HIV in T cells and macrophages. In human T cells, ddI is metabolized to the active moiety dideoxyadenosine-5'-triphosphate (ddA-TP) which acts at the level of reverse transcriptase. ddA-TP has a half-life of 12 or more hours in human T cells. As a result of this intracellular depot effect, it seemed possible that clinical antiviral activity might be attained even with twice daily dosing. Indeed, the results of this phase I trial suggest that this may be the case. This convenience of dosing is particularly advantageous in treating a disease such as HIV infection where therapy will probably have to be continued for years.

Another dideoxypurine, 2',3'-dideoxyadenosine (ddA) also has anti-HIV activity in T cells and macrophages with an activity profile similar to that of ddI. ddA is rapidly converted to ddI by the ubiquitous enzyme adenosine deaminase, so that for many purposes, ddA and ddI can be considered alternate forms of the same drug. Indeed, in an earlier clinical study, we found that we could detect ddI (but not ddA) in the serum of patients who were receiving ddA by the intravenous route. Like the patients in the present study, those patients were observed to have increases in T4 cells and declines in serum HIV p24 antigen (Robert Yarchoan, Neil R. Hartman, David G. Johns, and Samuel Broder, unpublished observations).

Both ddA and ddI are acid labile, and therefore are susceptible to degradation in the stomach. One of the breakdown products of ddA, adenine, is metabolized in the body to 2,8-dihydroxyadenine, which can cause renal toxicity. In contrast, ddI is degraded by acid to 2',3'-dideoxyribose and hypoxanthine, both of which are relatively non-toxic. For this reason we have focused our efforts into developing ddI for oral administration. In the present study, we found that ddI is reasonably well absorbed orally when given with antacids. It is worth stressing that effective buffering is crucial to obtain consistent absorption of ddI because of its acid lability. As an alternative approach, enteric formulations of ddI are now being studied (Neil Hartman, Robert Yarchoan, David Johns, and Samuel Broder, unpublished observation).

The 1 to 2 mg/dl increases in uric acid observed in the patients on the higher doses of ddI was most likely related to ddI catabolism to hypoxanthine and subsequently to uric acid. While hyperuricemia was not a clinical problem in

our study, it is conceivable that this could pose difficulties for patients on long term therapy or higher doses of ddI. It is possible that administration of allopurinol with ddI may reduce its catabolism to uric acid; however, other possible effects of allopurinol on ddI metabolism are at present unknown.

Overall, toxicity was minimal in this study, and some patients have now taken ddI for more than 6 months without adverse affects. Also, 5 patients who were previously on our ddA trial were subsequently given ddI (these patients are in addition to the 23 reported here), and some of these individuals have now taken ddA or ddI for a total of 11 months without developing toxicity. Of the possible ddI-related side effects seen in this study, the development of seizures in two patients who did not have structural CNS lesions is most noteworthy. Whether these were an effect of ddI or related to an underlying condition is at present not clear, and further clinical experience with ddI will be needed to resolve this point. Until that time, we would recommend that patients with a seizure history not take ddI and that caffeine or drugs known to induce seizures be avoided in patients taking ddI. Aside from these two patients, ddI-related toxicity was minimal, particularly considering that 9 of the patients on the study were intolerant of AZT. Only one patient developed bone marrow suppression, and this may have been related to his taking trimethoprim/sulfamethoxazole. Thus, this preliminary study suggests that ddI may be active against HIV in patients at doses which are well tolerated in the majority of patients.

It should be stressed that in an open study, it is difficult to determine whether an agent is truly inducing clinical improvement in patients with HIV infection, and it is not absolutely certain that increases in the number of T4 cells or declines in p24 antigen are predictive of an improvement of survival. The compilation of experience in anti-HIV therapy, however, suggests that improvement in these markers is likely to be associated with clinical benefit, and we would propose that ddI is worthy of testing in a larger controlled study. It may also be worthwhile to explore the use of ddI in combination with other anti-retroviral agents. As noted above, preliminary results of an alternating regimen of AZT and ddC suggest that a sustained anti-viral effect can be attained with reduced toxicity from either agent. Also, the results of Larder et al. suggest that strains of HIV which become resistant to HIV retain their sensitivity to several other dideoxynucleosides. At present, the issue of viral resistance remains a theoretical concern. However, as we find additional agents with clinical anti-HIV activity, we can begin to ask the question of whether a combination regimen will delay or prevent the development of drug resistance and whether as a result, patients will have a more sustained clinical response to therapy than is presently possible with AZT as a single agent.

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## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 07251-02 CO

## PERIOD COVERED

October 1, 1988, through September 30, 1989

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Phase I studies of ddC as a single agent or with AZT

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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## COOPERATING UNITS (if any)

## LAB/BRANCH

Clinical Oncology Program, Office of the Associate Director

## SECTION

## INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

## TOTAL MAN-YEARS

## PROFESSIONAL

## OTHER

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In previous annual reports, the first development of anti-retroviral drugs belonging to the dideoxynucleoside family was outlined. One such drug (AZT) has now achieved prescription status. Two others, ddC and ddA, have continued in our development effort. The goal is to find drugs which, alone or in combination, have a better therapeutic index than AZT alone. In a Phase I dose-seeking trial, we administered 5 dose regimens of 2',3'dideoxycytidine (ddC), a cytidine analogue with potent *in vitro* activity against human immunodeficiency virus (HIV) which had never previously been given to man, to 20 patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC). ddC was administered intravenously for 2 weeks and then orally for 4 or more weeks. ddC was well absorbed from the gut and crossed the bloodbrain barrier in these patients. Ten of the 15 patients who received 0.03 mg/kg to 0.09 mg/kg ddC every 4 hrs had increases in their absolute number of T4<sup>+</sup> T cells at week 2 ( $p < 0.05$ ); in many of the patients, however, these rises were not sustained. Eleven of 13 evaluable patients had a fall in their serum HIV p24 antigen by week 2 of therapy ( $p < 0.01$ ); in a few patients, the p24 antigen subsequently rose to baseline while in most the decline was sustained. Dose-related toxicities included a transient symptom complex of cutaneous eruptions, fever, and mouth sores; thrombocytopenia; and neutropenia. A late toxicity appearing after 6 to 14 weeks on ddC was reversible painful peripheral neuropathy. These results suggest that ddC has activity against HIV *in vivo* and has a different toxicity profile than 3'-azido-2',3'-dideoxythymidine (AZT). Based on these different toxicity profiles, we subsequently administered a regimen of AZT (200 mg orally every 4 hrs for 7 days) alternating with ddC (0.03 mg/kg every 4 hrs for 7 days) as a feasibility study. The regimen appears active and has less toxicity than either agent alone.

## INTRODUCTION

The chemotherapy of pathogenic human retroviral infections is rapidly evolving. 2',3'-dideoxycytidine (ddC), an analogue of 2'-deoxycytidine, belongs to a family of nucleoside analogues that includes 3'-azido-2',3'-dideoxythymidine (AZT). These agents were shown to have activity against human immunodeficiency virus (HIV) by Mitsuya and Broder in work discussed in previous annual reports, and one agent (AZT) has been proven to induce immunologic, virologic, and clinical improvements in patients with fulminant HIV infections. The use of AZT, however, is associated with substantial toxicity in some patients, most notably bone marrow suppression. Therefore, improved therapeutic regimens for the treatment of HIV infections are urgently needed.

Dideoxycytidine functions as a chain-terminating pyrimidine. The drug is anabolically phosphorylated (activated) in human T cells by a different pathway than the one involved in phosphorylating AZT. It is efficiently phosphorylated by human T cells and is approximately 10 times more potent than AZT on a molar basis at inhibiting HIV replication *in vitro*; complete inhibition in T cells is obtained at a concentration of 0.5  $\mu\text{M}$  of ddC under conditions of high multiplicity of infection. When fewer viral particles are used, ddC is effective at even lower concentrations (down to 10 nM). The drug, like all dideoxynucleosides under discussion, is active in monocytes (see accompanying annual report). ddC is resistant to deamination by cytidine deaminase (a ubiquitous enzyme which degrades many cytidine analogues including arabinosyl cytosine). In this study, we have administered ddC to patients with HIV infection in a Phase I study designed to test the feasibility of using this agent alone or in combination with AZT in patients and to determine the dose-limiting toxicity.

## MATERIALS AND METHODS

### Patients

20 male patients with HIV infection, ages 25 to 57, were entered into an initial trial of ddC. Nine of the patients had AIDS with a history of *Pneumocystis carinii* pneumonia (PCP), 8 had AIDS with Kaposi's sarcoma (KS), and 3 had AIDS-related complex (ARC). An additional 6 patients with HIV infection, ages 27 to 33, were subsequently entered into a pilot trial of AZT alternating with ddC; 2 of these 6 were AIDS patients who had had PCP, and 4 had ARC. One of the 6 patients had previously participated in the initial ddC trial. Each of the ARC patients in both trials had a history of oral candidiasis.

Overall, 23 of the patients were gay, one had used intravenous drugs, and had both risk factors for AIDS. Each patient had circulating antibodies to HIV, and each had less than  $350/\text{mm}^3$  helper T cells ( $\text{CD4}^+$  cells) when assayed prior to entry into the study. The patients were treated at the Warren G. Magnuson Clinical Center of the National Institutes of Health. The protocol was approved by the Institutional Review Board of the National Cancer Institute. Each subject gave informed consent prior to entry.

### Initial trial of ddC

ddC was synthesized for this study by the Developmental Therapeutics Program of the National Cancer Institute. Each patient received an intravenous test dose of

ddC administered over 1 hr, and some patients received an oral test dose the following day. The patients were then given ddC intravenously for 14 days according to the following regimens: 0.03 mg/kg every 8 hrs (Regimen A); 0.03 mg/kg every 4 hrs (Regimen B); 0.06 mg/kg every 4 hrs (Regimen C); 0.09 mg/kg every 4 hrs (Regimen D); or 0.25 mg/kg every 8 hrs (Regimen E). Each dose was administered over 1 hr. Patients who completed the intravenous therapy then received oral ddC at the same dose schedule for an additional 4 weeks. Patients who tolerated this initial 6 week period of therapy were given the option of continuing on oral ddC for an additional 6 to 8 weeks.

Patients were closely monitored for clinical and laboratory changes. They underwent testing for cutaneous delayed type hypersensitivity at entry and either at the end of 6 weeks or at the end of therapy (whichever came first) with 0.1 ml candida extract, intermediate strength (5 TU) purified protein derivative (PPD), tetanus toxoid, and trichophyton extract. Lymphocyte subsets reacting to Leu 3 (T4<sup>+</sup>, helper-inducer T cells) or to Leu 2 (T8<sup>+</sup>, suppressor-cytotoxic T cells) were analyzed by flow cytometry. Patients were also monitored for their ability to mount an in vitro proliferative response to tetanus, diphtheria, and A/ichi influenza virus antigens as previously described.

Heparinized plasma samples were obtained at various times after doses of ddC for measurement of ddC levels. In some patients, an aliquot of cerebrospinal fluid (CSF) was obtained by lumbar puncture during the second week of in vitro therapy. ddC concentrations in the plasma samples were measured by high-performance liquid chromatography using a modification of a method previously described. ddC concentrations in the CSF samples and simultaneously obtained plasma samples were measured by mass spectroscopy as described.

Serum samples obtained at entry and weekly during therapy were assayed for HIV p24 antigen using an enzyme-linked immunosorbent assay (ELISA) developed by Abbott Laboratories (Abbott Park, Illinois). Mitogen-stimulated peripheral blood mononuclear cells were cultured for HIV as previously described at entry, at 2 weeks, and at 6 weeks.

### Pilot study of AZT alternating with ddC

The AZT for this study was provided by Burroughs Wellcome Co. (Research Triangle Park, NC). After an initial evaluation, patients received a regimen of 200 mg of AZT orally every 4 hours for 7 day dosing periods, alternating with 0.03 mg/kg ddC orally every 4 hrs for 7 day dosing periods; the regimen was continued for 9 or more weeks. The patients were followed on an outpatient basis, and had weekly evaluations of their clinical, immunologic, and virologic status as described above except that viral culturing for HIV was not performed.

## Statistics

The statistical significance of the rises in T4 counts and the falls in HIV p24 antigen were assessed using the one-sided Wilcoxon signed rank test for paired observations.

## RESULTS

### Clinical Pharmacology

The peak ddC concentration was roughly proportional to the administered dose, and peak levels of 0.5  $\mu\text{M}$  (a dose providing complete protection against HIV *in vitro* under conditions of high multiplicity of infection), were attained with one-hr intravenous infusions of 0.06 mg/kg or greater. The average half-life of ddC was 1.2 hrs, and the oral bioavailability averaged 70-80%. Most of the drug appears to be eliminated by renal clearance. CSF samples obtained 2 to 3 1/2 hr after the initiation of a intravenous infusion contained an average of 20% (range 9 to 37%) of the concentration in simultaneously obtained plasma samples. Thus, ddC at least partially penetrated across the blood-brain barrier in these patients.

### Immunologic and Virologic Changes

There was no clear trend in the absolute number of T4 cells in the 4 patients receiving the lowest dose schedule of ddC. However, 10 of the 15 patients receiving the next three doses (0.03 mg/kg to 0.09 mg/kg every 4 hrs) had rises in their absolute number of T4<sup>+</sup> (helper-inducer) T cells by week 2; in these 15 patients, the mean number of T4<sup>+</sup> T cells rose from 85/mm<sup>3</sup> at entry to 117/mm<sup>3</sup> at 2 weeks ( $p < 0.05$ ). In addition, 12 of these 15 patients had increases in their ratio of helper-inducer/suppressor-cytotoxic T cells during the first 2 weeks ( $p < 0.01$ ). After week 2, however, there were decreases in the T4<sup>+</sup> T cells in some of the patients, and by week 6 (or end of therapy if that came earlier), the mean absolute number of T4 cells (90/mm<sup>3</sup>) was not significantly different from that at entry ( $p > 0.05$ ). Thus, patients receiving 0.03 to 0.09 mg/kg ddC every 4 hrs appeared to have transient increases in their T4 cells during the initial weeks of therapy.

In terms of immunologic function, 3 of the 17 patients who were anergic at entry developed positive skin tests to at least one antigen while on therapy. However, two patients (both on the 0.09 mg/kg every 4 hrs dose) who had weakly positive tests at entry lost their reactivity by week 6. The proliferative responses of peripheral blood lymphocytes to three recall antigens (influenza virus, diphtheria toxoid, and tetanus toxoid) was monitored in 15 of the patients. None had a substantial decline in their responses, and 6 had substantial improvement. In one patient (#19), there was a marked increase in the response to tetanus toxoid (3030 cpm of <sup>3</sup>H-thymidine incorporation at entry versus 24,990 cpm at week 3) which coincided with development of skin test reactivity to tetanus toxoid, and the increased proliferation may in part have been from immunization as a result of skin testing at entry. However, each of the other 5 patients with improved proliferation (#5, 9, 13, 16, and #18) responded to diphtheria toxoid (4 pts) or influenza virus (2 pts), and it is unlikely that this was the result of antigenic exposure during treatment.



There was no clear trend in the ability to isolate HIV in mitogen-stimulated cultures of peripheral blood mononuclear cells from the patients during therapy (data not shown). However, administration of ddC resulted in a decrease in serum HIV p24 antigen; of the 13 evaluable patients (i.e., those who had detectable HIV p24 antigen at some point during the study), 11 had declines during the first 2 weeks of therapy. In these 13 evaluable patients, the mean p24 antigen fell from 361 at entry to 135 pg/ml at week 2 ( $p < 0.01$ ). These decreases could not be accounted for by a change in the patients' serum antibody to p24 antigen. It is noteworthy that the falls in p24 antigen even occurred in the patients receiving the lowest dose tested. In 4 of the patients, there was a later rise in p24 antigen after these initial 2 weeks; however, at the end of the 6 weeks (or time when patients were taken off drug if earlier), the mean p24 antigen (173 pg/ml) was still less than baseline ( $p < 0.05$ ). Thus, although the patient number was small, ddC appeared to induce at least a transient fall in detectable serum p24 antigen in a significant subset of patients.

### Clinical Evaluation

In 9 patients, the initial administration of ddC was stopped before the completion of the first 6 weeks. In 2 patients (#6 and #15), this was because of pneumocystis pneumonia (diagnosed during the first 2 weeks); in 6 patients, because of drug toxicity (see below); and in one patient (#1), because of high fevers (the etiology of these was unclear). The most prominent toxicity during this initial therapy was a transient symptom complex of cutaneous eruptions, malaise, fever, aphthous mouth ulcers, and to a lesser extent arthralgias, ankle edema, and/or diarrhea which were present to some degree in 14 patients, particularly those on the higher doses. Some patients had a fall in serum albumin (averaging 0.5 gm/100ml) during this period, and one patient (#14) developed lip swelling. There was considerable variation among the patients in the severity and expression of this symptom complex. It generally appeared after two weeks of therapy (range 8 days to 4 weeks), and in each of the 7 cases in which the ddC was continued after the development of the complex, the symptoms subsided after 1/2 to 3 additional weeks on ddC.

Some patients receiving receiving the lower doses of ddC (up to 0.06 mg/kg every 4 hrs), developed mild transient thrombocytopenia which subsided even with continued drug administration. Three of the patients on the highest two doses developed more substantial thrombocytopenia and/or neutropenia which were dose-limiting toxicities. In contrast to patients receiving AZT in whom this is an early sign of marrow toxicity, the red blood cell mean corpuscular volume generally did not rise. Bone marrow examinations in 3 patients revealed erythroblastic vacuolization (2 patients) or no abnormalities (1 patient); megaloblastic changes were not prominent. Other toxicities, including hepatic, renal, or cardiac toxicity attributable to the drug, were not observed during this 6 week period.

A different toxicity, painful stocking-glove axonal sensorimotor peripheral neuropathy, developed in 10 of the patients who continued on ddC beyond the initial 6 weeks. It appeared to be dependent on the cumulative dose of ddC, generally appeared after 10 weeks except in the patients on the highest doses, and usually presented as a painful dysesthesia of the feet. Later, patients had

decreases in light touch, temperature, vibratory, and proprioceptive sensation, and in severe cases numbness, some weakness, and absent ankle jerks. Electrophysiological studies were consistent with axonal degeneration. This neuropathy is similar to the "painful sensory neuropathy" which can develop in patients with severe AIDS, and HIV-induced neuropathy may have contributed to the picture in some patients. The neuropathy in general worsened for up to 5 weeks after the drug was stopped, but then began to gradually improve both clinically and electromyographically.

Three patients (#2, #6 and #15) developed PCP while on ddC; two of these 3 cases appeared during the first 2 weeks of therapy. Other serious infections included one case each of cerebral toxoplasmosis (patient #7), progressive multifocal leukoencephalopathy (patient #13), and fatal gram negative pneumonia (organism could not be cultured) (patient #10). This last patient was not neutropenic at the time the pneumonia developed. In total, 5 patients (#2, #3, #7, #10, and #13) have expired at the time of this writing 12 months after the initiation of the protocol; all but 1 had  $<30$  T4 cells/mm<sup>3</sup> at entry.

Evaluation of clinical parameters of improvement in HIV-related symptomatology was somewhat complicated by the toxicities, particularly the fevers and the mouth sores which hampered eating. In spite of this, the patients gained an average of 0.5 kg during the first 6 weeks of the study; weight gain was particularly noticeable in the patients receiving 0.03 mg/kg every 4 hrs (mean increase 2.9 kg) and was not attributable to fluid retention. In addition, 6 of the patients reported increased energy or decreased fatigue while they were on ddC.

#### Pilot trial of AZT alternating with ddC

The results of this Phase I trial suggested that ddC had activity against HIV and also had a different toxicity profile than AZT. Also, because ddC appeared most active during the first 2 weeks of administration at the doses tested, we hypothesized that it might best be given on an intermittent basis; by administering ddC in an alternating schedule with AZT, we might also take advantage of their different toxicity profiles. To explore this approach, we administered a regimen of AZT (200 mg orally every 4 hours) alternating with ddC (0.09 or 0.18 mg/kg/day) to 18 patients with AIDS or ARC, with each drug being administered for 7 days at a time.

This study was started in June of 1987 and is still ongoing. The preliminary results from this study, however, do suggest that the drugs are better tolerated when administered in this manner than when either is given continuously. The first patient entered on the trial has now completed 85 weeks of therapy without developing either neuropathy or toxicity from AZT. Of the 18 patients entered, three developed moderate to severe neuropathy after 25 weeks or more of therapy; one of these patients had previously received continuous high-dose ddC on the earlier Phase I trial. Indeed, it appears that by administering ddC in an intermittent regimen, as compared to continuous administration, patients can tolerate at least two to four times as much cumulative drug without developing neuropathy. In addition, even in those patients in whom neuropathy developed, it generally subsided within several weeks after stopping the ddC. Hematologic toxicity was less than one would have expected from continuous therapy with AZT.

Overall, the patients had initial increases in their number of T4 cells (average increase 65 T4 cells/mm<sup>3</sup> at week 12 and 52 T4 cell/mm<sup>3</sup> at week 18). In addition, they had increases in their T4/T8 ratios, declines in serum HIV p24 antigen, and a greater than 4 kg average weight gain while on the regimen. Additional studies will be required to determine the role of ddC in this regimen (i.e. whether similar results would be obtained with intermittent AZT therapy) and whether this regimen is indeed superior to AZT as a single drug. The results do indicate, however, that reduced toxicity can be attained by alternate drug administration in this manner, and also that this approach is worthy of additional investigation.

## DISCUSSION

The results of this study demonstrate that ddC can be administered to patients with AIDS or ARC on a short-term basis; that serum drug levels above 0.5  $\mu$ M (an *in vitro* virustatic dose) can be attained; that the drug is well absorbed when administered orally; that it has straightforward pharmacokinetics; and that it penetrates into the cerebrospinal fluid. The study revealed several toxicities associated with ddC administration: a transient cutaneous eruption symptom complex; hematologic abnormalities (which also resolved in some patients continued on drug); and, after a number of weeks, peripheral neuropathy. The results also suggest that immunologic and virologic improvement can be detected in these patients, at least transiently, as a result of ddC administration. Finally, they suggest that an alternating regimen of ddC and AZT is reasonably well tolerated for up to 18 weeks and can confer immunologic and virologic improvements in patients with AIDS or ARC.

As already discussed, ddC is a member of a family of nucleoside analogues, dideoxynucleosides, several members of which are potent inhibitors of HIV replication *in vitro*. Another member of this family, AZT, was shown in initial studies to induce small immunologic improvements in patients with AIDS or ARC. In a subsequent double-blind placebo-controlled trial, AZT was shown to improve the survival of certain patients with AIDS. The T4 cell rises induced by AZT are often transient, however, and it causes substantial bone marrow suppression in many patients. A rise in the red blood cell mean corpuscular volume is an early manifestation of AZT toxicity, and it is thought that this megaloblastic marrow suppression results from AZT-induced depletion of thymidine-triphosphate levels.

Unlike AZT, ddC administration was not found to be associated with megaloblastic changes. However, as noted above, several unexpected toxicities were observed. The mechanisms responsible for these toxicities are unknown. ddC can be more efficiently phosphorylated in certain monocyte populations than is AZT, and it can form a choline adduct in mammalian cells; it is possible that one of these biochemical characteristics might contribute to the clinical picture. Of particular interest, and encouraging in a practical sense, was that the cutaneous eruption symptom complex cleared even with continued ddC administration. The later development of peripheral neuropathy, however, ultimately limited the time that ddC could be continuously administered as a single agent using these dose schedules. (Persons taking ddC in subsequent trials should probably avoid taking other neuropathic drugs, have their vibratory sensation monitored, and have the drug stopped, at least temporarily, when early symptoms develop.) It is possible that by understanding the pathogenesis of these reactions, we might be able to

prevent their occurrence while preserving the beneficial effects of ddC. The results of this study suggest that periodic drug-free intervals may significantly reduce ddC-associated neuropathy. Some patients have not been treated for more than one year without neurotoxicity.

The results of this study also showed that ddC induced at least transient (and in some patients more long-lasting) improvement in immunologic function and decreases in serum viral p24 antigen. However, ddC administration did not affect the ability to isolate HIV in mitogen stimulated cultures. This latter technique is probably not a useful indicator of an anti-HIV effect; it involves activating latently-infected cells and, unlike the serum p24 antigen, has not been shown to be affected by doses of AZT which are clinically beneficial. The decreases in p24 antigen seen in the patients were often abrupt and were observed even at the lowest dose of the drug that we tested. ddC appears to be an exceedingly potent agent, and our study provides a rationale for studying even lower doses of the drug. Indeed, preliminary results from an ongoing Phase I/II trial of ddC suggest that decreases of p24 antigen can be observed even in patients given 0.01 mg/kg every 4 hrs, and lower doses are now being tested. It is unclear why in some patients the level of p24 antigen rises after a nadir at 2 to 4 weeks (perhaps in some patients there is reduced phosphorylation of ddC after several weeks) and further research is needed to investigate this point.

The difference in toxicity profiles of ddC and AZT suggested that an alternating regimen utilizing both drugs might provide a sustained anti-retroviral effect with reduced toxicity as compared to either drug used alone; a secondary benefit of such a regimen might be that it would enable a repetition of the consistent anti-retroviral activity seen during the first weeks of ddC therapy. It appears that the hematological toxicity of AZT is drastically reduced by this approach.

We were concerned that the early ddC cutaneous symptom complex might recur during each week on ddC on such a regimen, but except in one patient, this did not occur. Indeed, the regimen tested was in general well tolerated. Also, it appeared to induce a later sustained increase in T4<sup>+</sup> T cells (comparable to that observed with AZT alone and a decline in HIV p24 antigen. It is too early to determine whether such a regimen will be better than AZT alone or indeed is substantially different than intermittent AZT administration per se (such a regimen has never been tested and might possibly provide an improved therapeutic index as compared to conventional AZT regimens); such questions will have to be addressed in larger controlled studies in which an alternating ddC/AZT regimen is compared to intermittent dosing with AZT.

In summary, the results of these trials suggest that although ddC is associated with some toxicity, it has activity against HIV in patients with AIDS or ARC even at the lowest dose tested and can be used in an alternating regimen with AZT. Additional studies will be needed to determine its role in the armamentarium against HIV infection.

#### Combination AZT and GM-CSF

We are also pursuing other forms of combination chemotherapy. During the past year, we have initiated a pilot study to test whether the growth-stimulating

hormone GM-CSF, given in an alternating regimen with AZT, can ameliorate the bone marrow toxicity of AZT without compromising the clinical activity of AZT. The starting dose of GM-CSF was 2 mg/kg per day. After a ten-day induction regimen of GM-CSF, AZT was alternated every other week with GM-CSF.

The results of this pilot study suggest that it is feasible to give AZT (200mg q4h) in a combination regimen with GM-CSF. About two-thirds of the patients developed a localized rash at the GM-CSF injection site. This was not a serious toxicity. About an equal number had some fever during the course of therapy. The fevers were easily controlled with non-steroidal anti-inflammatory agents. Other toxicities involved myalgias and arthralgias.

While the results are still preliminary, some patients have had increases in their T4 counts and other clinical parameters on this regimen. It is too soon to draw specific conclusions; however, the regimen appears to cause less neutropenia than standard-dose AZT as a single agent. Based on the observation by Dr. Perno that GM-CSF can potentiate the activity of AZT in macrophages, we are now exploring simultaneous administration of AZT and GM-CSF.

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## NOTICE OF INTRAMURAL RESEARCH PROJECT

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October 1, 1988, through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adenallene and cytallene active against HIV in vitro

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## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Adenallene [9-(4'-hydroxy-1',2'-butadienyl)adenine] and cytallene [1-(4'-hydroxy-1',2'-butadienyl)cytosine] can exert a potent antiviral activity against HIV-1 and HIV-2 in vitro. These two nucleoside analogues, which lack an oxacyclopentane can protect CD4<sup>+</sup> T-cells from the infectivity, replication, and cytopathic effect of HIV-1. In this report, we discussed that the antiviral effect of cytallene is readily reversed by 2'-deoxycytidine while that of adenallene is hardly reversed by 2'-dideoxyadenosine, the same features exhibited by 2',3'-dideoxycytidine and 2',3'-dideoxyadenosine respectively. We also discussed that adenallene was active against HIV-1 in monocytes/macrophages in vitro. These data may be of value in developing a new class of antiviral drugs for the therapy of HIV-related diseases.

## INTRODUCTION

Antiviral chemotherapy utilizing 3'-azido-2',3'-dideoxythymidine (AZT), one member of the oxacyclopentane ring-containing dideoxynucleoside family, has recently been proven to reduce the incidence of opportunistic infections in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex and confer a significant survival advantage to patients with established AIDS. The clinical effects of AZT appear to be correlated with an in vivo suppression of HIV replication by a reduction in p24 antigenemia. Antiretroviral chemotherapy, however, can be associated with substantial toxicity, particularly megaloblastic bone marrow suppression in the case of AZT, and new therapeutic modalities are urgently needed.

We have recently reported that adenallene and cytallene, nucleic acid base analogues which lack an oxacyclopentane moiety, could inhibit the infectivity, replication, and cytopathic effect of HIV-1 in vitro. In the current study, we further characterized antiviral activities and other physiological effects of adenallene and cytallene in vitro.

## MATERIALS AND METHODS

Viruses and Cells An HIV-1 preparation was obtained by ultracentrifugation of the culture supernatants of HTLVIIIg-producing H9 cells, and prepared to contain  $5.9 \times 10^{10}$  virus particles per ml. In the HIV cytopathic effect inhibition assay (vide infra), 0.5 virus particles per cell represented the minimum cytopathic doses of the virus preparations of HIV-1. A CD4<sup>+</sup> T cell clone (ATH8) and a normal CD4<sup>+</sup> tetanus toxoid-specific T-cell clone (TM11) as well as H9 cells were used as target cells for HIV. Characteristics of H9, ATH8, and TM11 cells have been described previously. Cell cultures were not synchronized as to cell-cycle. HIV-1<sub>pa-L</sub>, which was originally obtained from a sample of lung tissue (a kind gift from Suzanne Gartner and Mikulas Popovic), was expanded in normal peripheral blood monocytes and supernatant of the culture served as another source of infectious virus. To obtain a population enriched for monocytes/macrophages, two cycles of sheep erythrocyte (E) rosetting were performed as previously described. This isolation method yielded a non-E-rosetting population composed of 55-70% non-specific esterase-positive cells and 20-40% surface immunoglobulin-positive cells.

Reagents Adenallene [9-(4'-hydroxy-1',2'-butadienyl) adenine] and cytallene [1-(4'-hydroxy-1',2'-butadienyl)cytosine] were synthesized as previously described. All tested compounds were >95 % pure as shown by nuclear magnetic resonance spectra. Adenallene and cytallene are racemic mixtures [50% R and 50% S form (enantiomers)]. 2',3'-dideoxyadenosine (ddA) and 2',3'-dideoxycytidine (ddC) were provided by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, MD.

HIV Cytopathic Effect Inhibition Assay HIV cytopathic effect inhibition assays were performed as previously described. Target T-cells ( $2 \times 10^5$ ) were exposed to HIV for 1 hr, resuspended in 2 ml of fresh complete medium (CM) (RPMI 1640 supplemented with 4 mM L-glutamine, 15% undialyzed and heat-inactivated fetal calf serum, 50 units/ml of penicillin, and 50 ug/ml of streptomycin) containing 15%

(vol/vol) interleukin 2 (IL 2, lectin-depleted; Advanced Biotechnologies Inc., Silver Spring, MD) and 25 U/ml of recombinant IL 2 (Amgen, Thousand Oaks, CA), and the cells were incubated at 37°C in 5% CO<sub>2</sub>-containing humidified air. Control cells were treated similarly but were not exposed to the virus. At various time points, the number of viable cells was determined by the trypan blue dye exclusion method.

Determination of HIV-1 p24 gag Protein Expression H9 cells were exposed to polybrene, pelleted, exposed to HIV-1 (2,000 viral particles per cell), resuspended, and cultured with or without drugs at 37°C in 5 % CO<sub>2</sub>-containing humidified air. On day 3 of culture, the cells were extensively washed to remove cell-free virions, resuspended to 2x10<sup>4</sup> per ml, and cultured for 2 more days in the presence or absence of drugs. Culture supernatants were collected and the amount of p24 gag protein was assessed by enzyme linked immunosorbent assay (Du Pont, Boston, MA).

Monocytes/macrophages (1x10<sup>6</sup>) were exposed to 1 ml of 80,000 RT cpm/ml of HIV-1<sub>Ba-L</sub> preparation and resuspended and cultured in 4 ml of CM. On day eighteen in culture, the cells were extensively washed, resuspended to 4 ml of fresh CM. Five days after the washing, culture supernatants were collected and the amount of p24 gag protein was assessed by radioimmunoassay (Du Pont, Boston, MA).

## RESULTS

Inhibition of Replication of HIV-1 by Adenallene and Cytallene In Vitro We tested more than 30 acyclic nucleoside derivatives which lacked an oxacyclopentane moiety for in vitro anti-HIV-1 activity in our HIV cytopathic effect inhibition assay.

We found two acyclic nucleoside derivatives, adenallene and cytallene to exert a potent anti-HIV-1 activity in vitro. In the HIV-1 cytopathic inhibition assay, where 0.5 HIV-1 virus particles per cell represented a minimum cytopathic dose of the virus, a high multiplicity of infection (2,000 virus particles per cell) was employed. In the absence of drugs, by day 7 after exposure to HIV-1, almost all ATH8 cells were killed by the virus. However, 10 uM adenallene exerted a substantial protective effect on ATH8 cells, and at >50 uM, this compound gave a complete protective effect and enabled the cells to survive and grow. Another acyclic nucleoside analogue, cytallene, also exhibited a potent anti-HIV-1 activity in vitro. Cytallene at > 1.0 uM gave a virtually complete protective effect on ATH8 cells exposed to the virus. However, it was noted that the capacity of both adenallene and cytallene to nullify the cytopathic effect of HIV-1 was often lost by day 14 of culture, while the reference compounds ddA, ddC, and AZT remained effective against the virus through the 14-day period of time. The protective effects of adenallene and cytallene were confirmed in different target cells, cloned normal helper/inducer TM11 cells. These cells are specifically reactive to soluble tetanus toxoid antigen in the presence of appropriate accessory cells. TM11 cells had been stimulated by the antigen 6 days before and could grow in the presence of exogenous IL 2. In the absence of the drug, HIV-1 exerted a subatantial cytopathic effect on the TM11 by day 8 in culture, resulting in a profound decrease in the number of total viable cells.



However, the addition of 50 to 100  $\mu\text{M}$  of adenallene or the addition of 0.5 to 2  $\mu\text{M}$  of cytallene completely protected TM11 cells without affecting the growth of these normal cells. At 5  $\mu\text{M}$ , cytallene appeared to be somewhat more toxic to the cells than the reference compound, ddC.

Inhibition of HIV-1 gag Protein Expression by Adenallene and Cytallene When the  $\text{CD4}^+$  H9 cells, lymphoblastoid cells permissive for HIV-1 replication, were exposed to HIV-1 (2,000 virus particles per cells), more than 8 ng/ml of p24 gag protein was detected in the supernatant as determined by enzyme linked immunosorbent assay. In this system, adenallene, at 50  $\mu\text{M}$ , gave a marked reduction, and at 100  $\mu\text{M}$ , it completely suppressed the p24 gag protein production. Cytallene, at 0.1  $\mu\text{M}$  gave a partial protective effect, and at more than 1  $\mu\text{M}$ , it gave a virtually complete suppression of the p24 gag protein production.

Reversal of Anti-HIV Effect of Adenallene and Cytallene by 2'-Deoxynucleosides It has been shown that normal nucleosides reverse the anti-tumor or anti-viral effects of certain nucleoside analogues. For example, the addition of thymidine can readily reverse the anti-HIV effect of AZT in vitro. We therefore tested whether natural 2'-deoxynucleosides could reverse the anti-HIV effect of adenallene and cytallene. Adenallene, at 50  $\mu\text{M}$ , exerted a substantial protection against HIV-1. The addition of an equal molar or 2-fold higher concentration of 2'-deoxyadenosine (dAdo) did not reverse this antiviral effect. Five hundred micromolar dAdo gave a moderate toxicity to the growth of the cells and the reversal effect could not be assessed. However, assuming from the ratio of number of surviving ATH8 cells to that of virus-unexposed cells, it appeared that dAdo did not reverse the antiviral effect of adenallene. When we repeated this experiment three times using different concentrations of adenallene and dAdo, each time we obtained virtually the same data. The antiviral effect of adenallene was not reversed by 2'deoxyctidine (dCyd) either. In contrast, the addition of 1  $\mu\text{M}$  of dCyd to ATH8 cells protected by 1  $\mu\text{M}$  of cytallene against the virus, resulted in a substantial loss of the antiviral effect; and 50  $\mu\text{M}$  of dCyd almost completely reversed the antiviral effect of cytallene.

Reversal of Cytotoxicity of Adenallene and Cytallene by 2'-Deoxynucleosides We then asked whether normal nucleoside could reverse the toxicity of adenallene and cytallene against ATH8 cells. Adenallene, at 250  $\mu\text{M}$ , suppressed the growth of ATH8 cells with 60% reduction in the cell number as compared to the control no-virus, no-drug populations. The addition of an equal molar or 2-fold higher concentration of dAdo did not reverse the toxicity of adenallene. In contrast, the addition of 20 and 50  $\mu\text{M}$  of dCyd to 20  $\mu\text{M}$  of cytallene completely reversed the toxicity of cytallene.

Inhibition of HIV-1 p24 gag Protein Expression in Monocytes/Macrophages by Adenallene When peripheral blood monocytes/macrophages were exposed to HIV-1<sub>BR-L</sub>, a monocyto-tropic HIV strain, on day 23 of culture (5 days after washing procedure), 131.5 ng/ml of p24 gag protein was detected in supernatant. However, when the cells were cultured with 100  $\mu\text{M}$  of adenallene, the gag protein production was virtually completely suppressed. On day 23 of culture, no significant difference in viable cell number was observed with or without adenallene.

## DISCUSSION

To date, several anti-retroviral nucleoside derivatives have been shown to have activity against HIV-1 in vitro and/or in vivo. However, almost all such anti-retroviral nucleosides have an oxacyclopentane ring. We have recently reported that two acyclic nucleoside analogues, adenallene and cytallene, can exert a potent anti-HIV activity in vitro. We also described that the presence of two cumulated double bonds between the 1' and 2' carbons and between the 2' and 3' carbons required for the anti-HIV activity of these acyclic nucleoside derivatives. Furthermore, we learned that the specific placement of the hydroxyl group at the 4' carbon is critical for the in vitro anti-HIV activity suggesting that saturated acyclic analogues, like other nucleoside congeners, might require activation by successive phosphorylation to yield triphosphates by cellular enzymes.

The protective effect of cytallene is reversed by dCyd, as is the case with ddC. This observation suggests that cytallene functions as an analogue of dCyd and may be activated by 2'-deoxycytidine kinase. Indeed, we have observed that the toxic effect of cytallene for ATH8 cells can be completely reversed by the addition of dCyd. By contrast, it was apparent that the antiviral effect of adenallene could not be readily reversed by either dAdo or dCyd. We have observed similar results with ddA. In the case of ddA, there are several alternative pathways of anabolic phosphorylation, and perhaps the same is true for adenallene. These are topics for further research.

The antiviral activity of adenallene and cytallene against HIV in vitro could not have been easily predicted on the basis of previously known structure/activity relationships. We have recently obtained data that both adenallene and cytallene are stable at low pH. These drugs are resistant to pH 1 at room temperature and do not degrade for at least 16 hours. Such data might make them potentially suitable for regimens that involve prolonged therapy. Since from our experience with other nucleosides including AZT and ddC, adenallene and cytallene will probably be absorbed by oral administration. Taken together, our observation may be of value in developing a new class of experimental drugs for the therapy of HIV-related diseases.

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## NOTICE OF INTRAMURAL RESEARCH PROJECT

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October 1, 1988, through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

GM-CSF modulation of HIV and dideoxynucleosides in monocytes

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## TOTAL MAN-YEARS

## PROFESSIONAL:

## OTHER

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have investigated the influence of granulocyte-macrophage colony stimulating factor (GM-CSF) on the replication of HIV-1 in cells of monocyte/macrophage (M/M) lineage, and its effect on the anti-HIV activity of several 2'3'-dideoxynucleoside congeners of thymidine in these cells in vitro. We found that replication of both HTLV-III-Ba-L (a monocyctotropic strain of HIV-1) and HTLV-III-B (a lymphocytotropic strain) is markedly enhanced in M/M, but not in lymphocytes, exposed to GM-CSF in culture. Moreover, GM-CSF reduced the dose of HIV required to obtain productive infection in M/M. Even in the face of this increased infection, GM-CSF also enhanced the net anti-HIV activity of AZT and several related congeners: 2'3'-dideoxythymidine (ddT), 2'3'-dideoxy-2'3'-didehydrothymidine (D4T) and 3'-azido-2'3'-dideoxyuridine (AZddU). Inhibition of viral replication in GM-CSF-exposed M/M was achieved with concentrations of AZT and related drugs, which were 10-100 times lower than those inhibitory for HIV-1 in monocytes in the absence of GM-CSF. Other dideoxynucleosides not related to AZT showed unchanged or decreased anti-HIV activity in GM-CSF-exposed M/M. To investigate the possible biochemical basis for these effects, we evaluated the metabolism of several drugs in M/M exposed to GM-CSF. We observed in these cells markedly increased levels of both parent and mono-, di-, and triphosphate anabolites of AZT and D4T compared to M/M not exposed to GM-CSF. By contrast, only limited increases of endogenous competing deoxynucleoside-5'-triphosphate pools were observed following GM-CSF exposure. Thus, the ratio of AZTTP/dTTP and D4TTP/dTTP is several fold higher in GM-CSF-exposed M/M, and this may account for the enhanced activity of such drugs in these cells. Taken together, these findings suggest that GM-CSF increases HIV-1 replication in M/M, while at the same time enhancing the anti-HIV activity of AZT and related congeners in these cells. These results may have implications in exploring new therapeutic strategies in patients with severe HIV infection.

## INTRODUCTION

Human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS), infects and replicates within several types of human cells. Although early studies of this retroviral pathogen focused on CD4<sup>+</sup>T-lymphocytes as the principal target cell, there is increasing evidence that infection of cells belonging to the monocyte/macrophage lineage plays a crucial role in the pathogenesis and progression of this disease. In addition, since monocyte/macrophages (M/M) may differ from T-cells in their intracellular metabolism of drugs and their reaction to lymphokines, it is important that one consider the effect of antiretroviral therapeutic strategies on cells of the monocyte/macrophage lineage.

Our group has previously shown that 2'3'-dideoxynucleosides (ddN), a family of compounds lacking an hydroxy-group at the 3'-position of the sugar moiety, are potent *in vitro* inhibitors of HIV replication in both human T-lymphocytes and monocyte/macrophages. In addition, several members of this family of drugs have been shown to have clinical activity when administered to patients with severe HIV infection. One of these compounds, 3'-azido-2'3'-dideoxythymidine (AZT) prolongs life and improves neurologic abnormalities in certain patients with AIDS. The use of AZT, however, is associated with bone marrow suppression and other toxic side effects, particularly in patients with established AIDS, so that a recent focus of investigation has been directed toward developing strategies to overcome this problem. One approach currently under investigation is the use of granulocyte-macrophage colony stimulating factor (GM-CSF). GM-CSF is a cytokine that stimulates the maturation and differentiation of granulocyte- and monocyte-bone marrow precursor cells. In addition, Hammer et al have suggested that GM-CSF can inhibit HIV replication in U937, a CD4<sup>+</sup> cell line with certain monocytoïd properties. However, Folks et al. have found that GM-CSF may actually stimulate HIV replication in a subclone of U937.

Because of the interest in using GM-CSF in patients with HIV-related disease and the conflicting results obtained in monocytoïd cell lines, which may or may not be reliable biochemical models for tissue monocytes, we undertook an investigation of the *in vitro* effect of GM-CSF on HIV replication in purified human peripheral blood monocyte/macrophages, both as a single agent and in conjunction with dideoxynucleosides. Our results show that GM-CSF enhances HIV replication in fresh M/M by several hundred fold, and moreover, that infection of such stimulated cells can take place with lower concentrations of virus. In spite of this, the net potency of AZT and related dideoxythymidine analogues in inhibiting HIV infection is markedly increased in the presence of GM-CSF. This increase in activity appears to result from an enhanced cell-entry of drug and production of phosphorylated (activated) drugs in the face of minimal increases in the levels of the competing thymidine triphosphate. These results may have implications for the design of improved therapeutic strategies for patients with AIDS and related conditions.

## MATERIALS AND METHODS

Cells Monocyte-enriched peripheral blood mononuclear cells (PBMC) were obtained from healthy, HIV-negative donors using a Fenwal C3000 cell separator. The PBMC

were then further enriched for monocyte/macrophages (M/M) by elutriation as described by Gerrard et al. Monocyte/macrophages obtained by elutriation followed by 5-day culture were >95% non-specific esterase positive (Technicon Instruments Co., Tarrytown, NY), and more than 95% actively phagocytized 0.8u latex beads (Sigma Chemical Co., S. Louis, MO). In addition, they were <1% E-rosette positive, and 50% OKT4A positive (Ortho Diagnostic Systems, Inc., Raritan, NJ). Cell viability (as determined by trypan blue exclusion) was always greater than 95%. In some experiments, H9, a T4-positive lymphocytic cell line, was used as control. Also, in certain experiments, enriched lymphocyte populations were obtained by elutriation followed by incubation on plastic dishes to remove adherent cells. These cells were <2% non-specific esterase positive. It should be noted that the control M/M used in this study (i.e. elutriated cells cultured for 5 days prior to exposure to HIV) are different than the M/M populations which formed the core of our previous paper on the effect of dideoxynucleosides on HIV infection in M/M.

Virus A monocyctotropic strain of HIV-1, HTLV-III<sub>Ba</sub>-L (kindly provided by Drs. S. Gartner and M. Popovic), and a lymphocytotropic strain, HTLV-III<sub>g</sub> (ElectroNucleonics Laboratory Inc., Silver Spring, MD), were used in these experiments, as previously described.

Drugs 3'-azido-2'3'-dideoxythymidine (AZT) (Wellcome Research Laboratories, Research Triangle Park, NC), 2'3'-dideoxycytidine (ddC), 2'3'-dideoxythymidine (ddT), 2'3'-dideoxyadenosine (ddA), 2'3'-dideoxyinosine (ddI) (Pharmacia Fine Chemicals, Piscataway, NY), 2'3'-dideoxy-2'3'-didehydrothymidine (d4T) (supplied by Developmental Therapeutics Program, DCT, NCI, Bethesda, MD), and 3'-azido-2'3'-dideoxyuridine (AZddU) were diluted in distilled water and kept at 4° until used. Human recombinant GM-CSF (Sandoz Research Inst., East Hanover, N.J.) was reconstituted in distilled water at 500,000 U/ml stock solution, and stored at 4° until used. Our GM-CSF preparation contains  $5.6 \times 10^6$  CML (chronic myelogenous leukemia) units per milligram of glycoprotein, as measured by minor modifications of a rapid proliferation CML assay previously published by Griffin et al. [<sup>3</sup>H]-AZT (sp. act. 3 Ci/mmol), and [<sup>3</sup>H]-d4T (sp. act. 10 Ci/mmol) (Moravsek Biochemical, Brea, CA), were freeze-dried and used immediately after reconstitution.

Antiviral drug assay The tests of antiviral activity were performed with minor modifications of a previously published procedure. Briefly, 10<sup>5</sup> purified elutriated monocyte/macrophages were seeded at day -5 in 1 cm<sup>2</sup>-well of a 48 well-plate (Costar, Cambridge, MA), and cultivated in RPMI 1640 medium (Gibco Lab, Grand Island, NY) supplemented with 20% heat-inactivated fetal calf serum (FCS) (Hyclone Laboratories Inc., Logan, UT), 2mM L-glutamine, 50 U/ml penicillin and 50 ug/ml streptomycin (Gibco) (complete medium) for 5 days in the presence or absence of 100 U/ml of GM-CSF. Unless stated otherwise, cells were treated from day -5 with GM-CSF and then cultured in the continuous presence of this cytokine. Cells, viruses and FCS were tested for mycoplasma contamination, and found negative. At day 0, M/M were preexposed for 20 min to various concentrations of drugs, and then challenged with HTLV-III<sub>Ba</sub>-L (80,000 cpm/ml reverse transcriptase) as previously described. This is at least 10 times the minimum infective dose in 5-day cultured M/M not exposed to GM-CSF.

M/M for 5 days before exposure to virus and drugs is thus an important variable that should be taken into consideration. M/M exposed continuously to 100 U/ml GM-CSF showed a high titer of viral production (at least 10 times more than without this cytokine), consistently detectable by day 5. Viral production was sustained for at least 35 days. No substantial enhancement of viral production was seen in most experiments using lower concentrations of GM-CSF, i.e. 1 and 10 U/ml. Since GM-CSF induces proliferation of monocytes, we wondered whether the increase in viral production was simply due to the GM-CSF-induced increase in cell number. However, the number of cells 7 days after viral challenge ranged up to 20 fold higher for M/M exposed to GM-CSF as compared to unexposed cells, whereas the increase in p24 production at day 7 was about 100-1,000 fold.

Furthermore, in early experiments, we tested the minimum viral dose capable of inducing consistent and productive infection in M/M. We found that while the minimum HTLV-III<sub>Ba</sub>-L infective dose for untreated M/M was 8,000 cpm/ml of RT, as little as 80 cpm/ml of the same virus preparation induced viral infection in GM-CSF-treated M/M. Thus, it is unlikely that the increase in cell number per se caused by GM-CSF accounts for the major enhancement of viral production observed in our studies. Syncytia formations were easily detected, starting from day 9, in GM-CSF-M/M exposed to HTLV-III<sub>Ba</sub>-L. At this time, multinucleated giant cells were seen in culture, in association with an inhibition of cell replication. From day 20, giant cells became pycnotic. However, they maintained their ability to produce large amount of virus at least up to day 35. In one experiment, we subjected HIV-exposed GM-CSF-stimulated M/M to 3 cycles of freezing and thawing to explore whether this would release additional viral particles which might be sequestered within intracytoplasmic vacuoles; however no increase in HIV-p24 antigen was observed.

M/M treated with GM-CSF only before virus challenge (that is from day -5 to day 0), followed by extensive washing to remove this cytokine, initially gave rates of viral production comparable to those seen in cells continuously exposed to GM-CSF. After day 14, however, a substantial waning in viral production was seen, and from day 35 after viral challenge this level became similar to the viral yield obtained in GM-CSF-unexposed M/M. Viral production in M/M treated with GM-CSF starting from day 0 was less enhanced than that observed with continuous treatment from day -5. In some experiments, we infected blood-derived M/M that had been allowed to mature for 5 days on plastic dishes, further purified by adherence, and then exposed to GM-CSF. GM-CSF did not have any substantial effect on HIV replication in these 5-day adherent M/M shown), possibly because of the relatively low number of high-affinity GM-CSF receptors in mature macrophages. This is a topic for further research.

In similar experiments, we exposed elutriated M/M cultivated for 5 days in the presence or absence of GM-CSF to HTLV-III<sub>B</sub>, a lymphocytotropic viral strain. In agreement with our previous experience, in the absence of GM-CSF we obtained inconsistent viral replication using this strain; productive infection was attained in only 3 of 6 experiments using M/M not exposed to GM-CSF, and in the productive cultures there was a delayed, lower peak of viral production as compared to the results obtained using the monocytotropic strain, HTLV-III<sub>Ba</sub>-L. However, HTLV-III<sub>B</sub> replication in M/M exposed to GM-CSF was consistent and easily detectable starting from 7 days after virus exposure, with a peak of viral

production after 14-21 days of culture about 20 times greater than the maximum level obtained without GM-CSF. We also tried to define the minimum infective dose in these cells. We observed that in the absence of GM-CSF, at least 100 times more HTLV-III<sub>B</sub> was required to bring about a productive infection of M/M, compared to the dose of HTLV-III<sub>B</sub> required to do so in the presence of GM-CSF. Thus, in our hands GM-CSF induced in M/M an enhancement of HTLV-III<sub>B</sub> replication in conjunction with a substantial reduction (at least 100 fold) of the minimum infective dose of HTLV-III<sub>B</sub>, an otherwise non-monocytotropic strain of HIV-1.

Also consistent with our previous report (8), AZT induced inhibition of HTLV-III<sub>Ba</sub>-L replication in M/M precultivated for 5 days in media alone. Over 90% suppression of viral replication was obtained with 1  $\mu$ M AZT, and 23% suppression was obtained with 0.1  $\mu$ M. Interestingly, despite the substantial increase in HIV production induced in such cells by GM-CSF alone, AZT was much more potent in inhibiting viral replication in the presence of this cytokine; >98% inhibition of viral replication was achieved with 0.1  $\mu$ M AZT, and even with 0.01  $\mu$ M AZT, about 90% viral suppression was observed (this was not due to an artifact of increased cell death, a point we will address later in this article). This effect was not seen in fresh, monocyte-depleted lymphocytes; in these cells, 10  $\mu$ M AZT completely inhibited HIV-1/HTLV-III<sub>B</sub> replication in the absence of GM-CSF, and neither stimulation of viral production nor enhancement of antiviral activity of AZT was seen with exposure of lymphocytes to 100 U/ml GM-CSF. This is in agreement with the results obtained by Walker et al, in which no substantial amount of GM-CSF receptors could be detected on T-lymphocytes.

To determine whether GM-CSF enhancement of antiviral activity in M/M was specific for AZT, or was also seen with other 2'3'-dideoxy-analogues of thymidine, we tested the effect of GM-CSF on three AZT congeners, 2'3'-dideoxythymidine (ddT), 2'3'-dideoxy-2'3'-didehydrothymidine (D4T), and 3'-azido-2'3'-dideoxyuridine (AZddU) (although AZddU is in fact a uridine congener, it is phosphorylated by thymidine kinase and in this sense acts as congener of AZT). We also studied three other 2'3'-dideoxynucleosides, 2'3'-dideoxycytidine (ddC), 2'3'-dideoxyadenosine (ddA) and 2'3'-dideoxyinosine (ddI), drugs with different bases and which are phosphorylated in human cells by different kinases compared to AZT. With each of the AZT congeners, there was an enhancement of antiviral activity in the presence of GM-CSF. Consistent with our previous results, the ED<sub>50</sub> for AZT in elutriated M/M cultured for 5 days was roughly 0.1  $\mu$ M, while it decreased down to between 0.001 and 0.01  $\mu$ M when these M/M were exposed to GM-CSF. ddT itself induced complete viral inhibition at 10  $\mu$ M in elutriated M/M, the ED<sub>50</sub> being 1  $\mu$ M. This result is remarkable inasmuch as ddT does not have strong anti-HIV activity in T-cells in our hands. However, in the presence of GM-CSF, the ED<sub>50</sub> was lowered to 0.1  $\mu$ M. In a similar manner, the ED<sub>50</sub> of D4T was between 0.1-1  $\mu$ M for untreated M/M, and 0.01-0.1  $\mu$ M for GM-CSF-exposed M/M. Finally, AZddU was only partially effective in our hands in elutriated M/M after 21 days of culture, even at the highest concentrations tested (about 60% protection at 100  $\mu$ M after 21 days of culture). However 10  $\mu$ M AZddU induced complete viral suppression in GM-CSF-treated M/M, with an ED<sub>50</sub> of 0.1-1  $\mu$ M. These data were confirmed in two different experiments by determination of RT activity in culture supernatants; the results were substantially parallel to those obtained measuring HIV-p24 antigen production. We also tested the ability of AZT and its congeners to inhibit syncytia formation. Low concentrations of AZT and ddT completely



inhibited syncytia induced by HIV-infection in GM-CSF-exposed M/M; similar effects were seen with D4T and AZddU.

We asked whether GM-CSF had an effect on viral infection and/or on antiviral activity of AZT in H9, a T-lymphocytic cell line. Neither increase of viral production nor enhancement of antiviral activity of AZT and related congeners was seen, in agreement with data obtained with AZT in fresh lymphocytes. Finally we exposed M/M to the lymphocytotropic strain HTLV-III<sub>B</sub> in the presence or absence of GM-CSF. The results are substantially parallel to those obtained with the monocytotropic strain HTLV-III<sub>Ba-L</sub>, showing an overall potentiation of viral suppression by AZT and related congeners in M/M exposed to GM-CSF. Thus, the results indicated that AZT and each of the closely related congeners tested are 10 or more times more active at suppressing replication of two different strains of HIV in GM-CSF-exposed M/M than in M/M not exposed to GM-CSF.

By contrast, when we tested the effect of GM-CSF on other dideoxynucleosides not related to AZT, we found that there appeared to be some reduction of anti-HTLV-III<sub>Ba-L</sub> activity in fresh M/M in the presence of GM-CSF. Comparable results were obtained using the other HIV-1 strain, HTLV-III<sub>B</sub>. Our data do not distinguish whether this represented unchanged anti-HIV activity in the presence of an increased efficiency of viral replication, or alternatively, an actual reduction of anti-HIV activity. In either case, the results indicate that GM-CSF seems to enhance the anti-HIV effect of AZT and its congeners in M/M, while the effect of several other dideoxynucleosides is unchanged or possibly even reduced in M/M cultured with this cytokine.

To determine whether the enhanced antiviral activity of AZT and its congeners by GM-CSF might be due to an increased cell toxicity, we assessed this parameter related toxicity, as assessed by trypan blue exclusion, at concentrations up to by two different methods. In the absence of GM-CSF, M/M failed to show any drug related toxicity, as assessed by trypan blue exclusion, at concentrations up to 100  $\mu$ M AZT, 500  $\mu$ M ddT, 10  $\mu$ M D4T, or 100  $\mu$ M AZddU. Also, no inhibition of phagocytic activity was seen at the same concentrations. In the presence of GM-CSF, phagocytosis was substantially unaffected by drugs. However, high concentrations of AZT and AZddU did lower the number of M/M exposed to GM-CSF in a dose-dependent fashion; in the case of AZT, a 50% reduction of viable M/M was obtained at 10  $\mu$ M. On the other hand, 1  $\mu$ M AZT was devoid of cytotoxicity in monocytes exposed to GM-CSF, and this concentration is >100 times the ED<sub>50</sub> described earlier under these conditions. Similarly, ddT and D4T did not substantially affect cell viability at concentrations up to 500  $\mu$ M and 10  $\mu$ M respectively. Thus, while GM-CSF does have an effect on the toxicity of AZT and analogs at the same time as it increases their antiviral activity, >95% HIV suppression by AZT analogues in GM-CSF-treated M/M is obtained at concentrations 10-500 times (depending on different AZT analogs) lower than those necessary to induce such viral inhibition in HIV-exposed T-lymphocytes; moreover, in the case of AZT, little or no toxicity is observed at drug concentration typically attained in vivo (1-3  $\mu$ M).

To evaluate some possible mechanisms responsible for the interaction of GM-CSF and AZT congeners in fresh M/M cultivated for 5 days, we studied the metabolism of AZT and D4T in M/M with or without GM-CSF. In the absence of GM-CSF, M/M had

low levels of intracellular AZT-5'-triphosphate (AZTTP). GM-CSF does not eliminate the relative block at the level of thymidylate kinase which is characteristic for AZT metabolism. Exposure of the M/M to GM-CSF dramatically increased the intracellular levels of both parent AZT and its mono-, di-, and triphosphate anabolites. In particular, AZTTP level increased more than 15 fold. These effects were not seen in H9 T-cell line exposed to GM-CSF. In part, the increase in AZTTP in M/M exposed to GM-CSF may be due to an increase in the cell entry of AZT, as evidenced by the higher intracellular levels of AZT as parent compound. Also, GM-CSF may have an effect on the level of intracellular thymidine kinase. The activity of this enzyme, which catalyzes the initial phosphorylation of AZT and related congeners, and thymidine as well, is very low in M/M when compared to T-cells. M/M exposed to GM-CSF had approximately a two-fold increase in thymidine kinase compared to control M/M cultured without GM-CSF. A similar increase in phosphorylation was obtained with D4T: active metabolites of D4T were barely detectable in M/M unexposed to GM-CSF, whereas large amounts of both D4T and its mono-, di-, and triphosphate moieties were detected in GM-CSF-exposed M/M. Indeed, the levels in such cells were comparable to those seen in H9 T-cells.

We also studied the endogenous 2'-deoxythymidine-triphosphate (dTTP) pool, which competes with AZTTP and related congeners at the level of viral reverse transcriptase. We found that the concentration of dTTP was very low in this population of M/M, as previously reported (8) for M/M tested under different conditions, and dTTP increased less than two fold in M/M exposed to GM-CSF for 5-7 days. In the current work, we observed that elutriated M/M cultured for 5 days in media alone have a very low level of endogenous dNTP's compared to the levels obtained in other M/M populations. Thus, GM-CSF-exposed M/M have a substantially higher ratio of AZTTP/dTTP than control M/M, a result which might explain the increased activity of AZT and related congeners in such cells.

## DISCUSSION

In this report, we have found that GM-CSF activates the replication of HIV-1 in fresh M/M cultured for 5 days, yet at the same time it potentiates the anti-HIV activity of AZT and related congeners in fresh M/M. Furthermore, we show that this effect on AZT may be accounted for by a preferential enhancement of AZT cell entry and subsequent phosphorylation induced by GM-CSF. Similar considerations apply to other dideoxy-analogues of thymidine.

We wish to stress that any specific clinical or therapeutic inference from our data should be made only with great caution at this time. Nevertheless, the infection of cells belonging to the monocyte lineage is increasingly recognized as an important event in the pathogenesis of AIDS. Monocyte/macrophages are currently believed to be one of the earliest cells infected after exposure to HIV, and may play an important role in spreading HIV throughout the immune system. In addition, monocyte-derived cells appear to be the primary target for HIV-infection of the nervous system. Thus, factors which influence the degree of replication of HIV in M/M may influence the course of HIV infection in patients. While certain strains of HIV have been reported to replicate in peripheral blood monocytes in the absence of exogenous stimulation, we show here that stimulation of these cells by GM-CSF markedly enhances their capacity to permit replication

of HIV. Indeed, even the lymphocytotropic strain of HIV-1, HTLV-III<sub>B</sub>, which in our hands does not replicate well in unstimulated M/M, replicates quite efficiently in M/M exposed to GM-CSF. It is conceivable that in our system, GM-CSF stimulates the production of host-cell transcriptional factors which in turn can potentiate viral transcription or post-transcriptional events.

It is of note that another marrow growth factor, macrophage-colony stimulating factor (M-CSF or CSF-1) has been shown by Gendelman et al. to enhance HIV production by M/M. In the case of CSF-1-stimulated M/M, however, most of the HIV production was reported to be sequestered in vacuoles, while with GM-CSF abundant virus was released into the media. (Even though we failed to find evidence of an increase in p24 released into the media by disrupting GM-CSF-exposed M/M using three cycles of freeze-thawing, this would not completely exclude the possibility that GM-CSF induced a limited form of virus sequestration in intracytoplasmic vacuoles; to specifically address this point, one would have to examine GM-CSF-exposed HIV-infected M/M by electron microscopy).

It is of interest that GM-CSF treatment of target M/M potently enhances the replication of the lymphocytotropic strain HTLV-III<sub>B</sub> in these cells; in this context GM-CSF has a net effect of converting a lymphocytotropic virus to a monocytotropic virus. Thus, the available data from this and other studies suggest that GM-CSF is, if anything, an even more potent stimulus for HIV replication in M/M than CSF-1. GM-CSF is produced by a number of cells in vivo. As one such cell is the T-lymphocyte, it is possible that GM-CSF might play a central role in the progression of HIV infection to AIDS. Antigenic stimulation of T-cells may lead to GM-CSF production, which may then lead to enhanced HIV replication in macrophages and, in turn, spread of virus to more T-cells.

At the same time, GM-CSF markedly enhances the anti-HIV effect of AZT and closely related drugs in monocyte/macrophages. It appears to do so by substantially increasing intracellular AZT-5'-triphosphate (AZTTP) levels, while exerting only a slight effect on the levels of competing endogenous thymidine-triphosphate (dTTP). To some degree, the enhanced phosphorylation of AZT may be due to a GM-CSF-induced increase in thymidine kinase (which not only catalyzes the initial phosphorylation of thymidine and related ddN analogues, but also is reported to act on AZddU). However, this does not appear to be the only mechanism responsible. M/M stimulated with GM-CSF and exposed to AZT have substantially higher levels of intracellular AZT as unphosphorylated compound than unstimulated M/M, suggesting that GM-CSF may also selectively enhance the cell entry of AZT and related drugs into M/M. It is worthwhile to point out that AZT and its normal counterpart, 2'-deoxythymidine, very likely permeate the cell membrane of lymphocytes and erythrocytes via different mechanisms; thus, lymphokines may have differential effects on the entry of different nucleosides. Also, in the case of the physiologic deoxynucleoside-5'-triphosphate, some of the endogenous thymidine pools may depend heavily on de novo synthesis from dUMP, and be regulated by feedback mechanisms. The physiologic 2'-deoxynucleotide may therefore not be affected in precisely the same way as those arising from ddN, by changes in membrane transport or diffusion. Additional studies will be required to detail the mechanism(s) responsible for the effect of GM-CSF on the phosphorylation of AZT and related analogues, and the mechanisms responsible for the reduced antiviral activity of ddN not related to AZT in GM-CSF-exposed M/M.

We are presently in the process of evaluating how exposure of M/M to GM-CSF affects the metabolism of another dideoxypyrimidine, ddC, and a dideoxypurine, ddA, in order to delineate the basis for the differential effects observed on anti-viral activity. Preliminary results indicate that by contrast to the >10-fold enhancement of AZTTP induced by GM-CSF, there is only a two-fold enhancement of ddCTP. The competing physiologic nucleotide, 2'-deoxycytidine-5'-triphosphate (dCTP), however, was below the limit of detection of our assay in both of these cultured populations of M/M, and for this reason, we could not calculate the effect of GM-CSF on the ddCTP/dCTP ratio. Also, while ddATP and dATP were measurable in M/M exposed to GM-CSF, they were below the limit of detection in the media-control (unstimulated) M/M. Thus, while these preliminary results suggest that the increase in ddCTP induced by GM-CSF was substantially less than the increase of AZTTP, and that the increase of ddATP was likely coupled with a substantial increase in the competing dATP, additional experiments will be needed to fully characterize the effects of GM-CSF on the intracellular biochemistry of these dideoxynucleosides. Nevertheless we can speculate that the limited net activation of metabolism of such non-thymidine ddN is probably not sufficient to overcome the dramatic increase in viral yield induced by GM-CSF stimulation.

GM-CSF is currently being studied as a supplementary drug for the treatment of AIDS because of the possibility that it may counteract the marrow suppression induced by HIV infection or by AZT administration. The results reported here suggest that one may wish to closely monitor patients when using GM-CSF in the setting of HIV-infection since it might enhance viral replication. However, as also shown here, the simultaneous administration of GM-CSF with AZT, D4T, or related analogs may induce a potent antiviral effect in monocytes. Could such a combination also increase AZT toxicity in vivo in marrow blood-forming cells? The answer is not known; however, a recent study suggested that in contradistinction to our results obtained in M/M, GM-CSF might reduce the phosphorylation of AZT in bone marrow cells while increasing the levels of thymidine triphosphate. It is thus conceivable that GM-CSF may act to increase the overall therapeutic index of AZT and related thymidine analogues. The simultaneous administration of these two agents, therefore, may be worth exploring in patients with severe HIV infection.

#### PUBLICATION

1. Perno C-F, Yarchoan R, Cooney DA, Hartman NR, Webb SA, Hao Z, Mitsuya H, Johns DG, Broder S. Replication of human immunodeficiency virus in monocytes. Granulocyte/macrophage colony-stimulating factor (GM-CSF) potentiates viral production yet enhances the antiviral effect mediated by 3'-azido-2'-deoxythymidine (AZT) and other dideoxynucleoside congeners of thymidine. *J Exp Med* 1989;169:933-51.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

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## PERIOD COVERED

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## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dextran sulfate suppression of viruses in the HIV family

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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## SECTION

## INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

## TOTAL MAN-YEARS

## PROFESSIONAL

## OTHER

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The first step in the infection of human T-lymphocytes by human immunodeficiency virus (HIV) is attachment to the target cell receptor, which consists of the CD4 antigen as an essential component. The process of specific binding between the CD-4 receptor and the viral envelope glycoprotein may be vulnerable to attack by antibodies, chemicals, or small peptides. Dextran sulfate, a long chain polymer of glucose with a molecular weight of about 8,000, containing 17-20% sulfur, was found to block the binding of virions to various target CD4<sup>+</sup> T-lymphocytes, inhibit syncytia formation, and exert a potent inhibitory effect against HIV-1 at concentrations that do not suppress the growth and functions of T-cells in vitro. Another anionic polysaccharide, pentosan sulfate with a molecular weight of about 6,000, containing 24% sulfur, was also found to block viral binding and exerted a potent antiviral activity against HIV. Dextran sulfate and pentosan sulfate might be viewed as prototypes for a large class of anionic polysaccharides, from both natural and synthetic sources, which can block viral replication by inhibition of the viral binding in vitro.

## INTRODUCTION

In the relatively short time since the first clinical recognition of acquired immunodeficiency syndrome (AIDS), a great deal has been learned about the life-cycle of human immunodeficiency virus (HIV) as well as the treatment of the diseases it causes. Antiviral chemotherapy directed against reverse transcriptase utilizing 3'-azido-2',3'-dideoxythymidine (AZT), has recently been proved to reduce the incidence of opportunistic infection in patients with AIDS and AIDS-related complex and confer a significant survival advantage to patients with established AIDS. The use of AZT, however, can be associated with substantial toxicity, particularly megaloblastic bone marrow suppression. Improved therapeutic regimens and other therapeutic modalities for the treatment of HIV-1 infection are urgently needed.

Dextran sulfate, a long chain polymer of glucose with a molecular weight of about 8,000, containing 17-20% sulfur, has recently been shown to be a potent anti-HIV-1 agent *in vitro*. Several groups, including those headed by Marin Moelling in Berlin and Erik DeClercq in Leuven have provided insights into the possible application of this drug in retroviral infections. This compound has been given orally to human beings for more than two decades as anti-coagulant or anti-lipemic agent. We found that dextran sulfate can block the binding of HIV to CD4<sup>+</sup> T cells, inhibit syncytia formation, and exert a potent antiviral activities against HIV *in vitro*. We also found that another anionic polysaccharide, pentosan sulfate (xylanpolyhydrogen sulfate; Hoe/Bay 946) can block the binding of HIV and exert an antiviral effect against HIV.

## MATERIALS AND METHODS

### Viruses and Anionic Polysaccharides

HIV-1 and HIV-2 were pelleted by ultracentrifugation from the culture supernatants of HTLV-III<sub>B</sub>-producing H9 cells and HIV-2-producing CEM cells, respectively. Dextran sulfate and dextran (M.W. 9,400) were obtained from Kowa Pharmaceutical, Japan. Pentosan sulfate was kindly provided by Hoechst Aktiengesellschaft, West Germany.

### Assay of Inhibition of HIV Infectivity

HIV cytopathic effect inhibition assay was performed as previously described. Briefly,  $2 \times 10^5$  target T cells were exposed to HIV, and cultured in the presence or absence of various concentrations of drugs. Cells were continuously exposed to the drug. Control cells (without virus) were treated similarly but were not exposed to the virus. At various time points, total viable cells were counted. Techniques for Southern blot hybridization were as described.

### Assay for Inhibitory Effect of Dextran Sulfate on HIV-1 Reverse Transcriptase and Mammalian DNA Polymerase alpha

Five microliters of purified HIV-1 reverse transcriptase or mammalian DNA polymerase alpha was incubated at 37°C using poly (rA)•(dT)<sub>12-18</sub> or poly(dA)•

(dT)<sub>12-18</sub> as a template in the presence or absence of various concentrations of drugs, as previously described.

#### Assay for Inhibition of Syncytia Formation

Half million of ATH8 cells were cultured with or without an equal number of chronically HIV-1-infected H9 cells in Costar 12 well-culture plate, following 2 hr pre-incubation with or without drugs. In 48 hr of co-culture, number of giant cells was assessed on the inverted microscope.

#### Assay for Inhibition of HIV-1 Binding

Assay for inhibitory effect of HIV-1 binding was performed as previously described. Briefly, tritiated virus was prepared by incubating two-week-infected H9 cells with <sup>3</sup>H-uridine for 48 hours, pelleting the cell-free supernatant, and resuspended. Labelled virus was diluted on the basis of titrations to give 50,000-100,000 cpm bound/ml, 10 ul added to  $2 \times 10^5$  H9 cells, the cells washed, lysed with distilled water, transferred to scintillation vials, and counted. Specific inhibition was calculated according to the formula: % Specific inhibition =  $100 \times (\text{CPM}_{\text{control}} - \text{CPM}_{\text{sample}}) / (\text{CPM}_{\text{control}} - \text{CPM}_{\text{max}})$  where  $\text{CPM}_{\text{max}}$  is the radio-activity observed with MOKT4A (65-89% inhibition at the concentrations used),  $\text{CPM}_{\text{control}}$  the radioactivity in the absence of drug or antibody, and  $\text{CPM}_{\text{sample}}$  the observed experimental radioactivity. Non-specific binding to cells not expressing the CD4 antigen was negligible at dilutions used. Determinations were performed in triplicate.

### RESULTS

#### In Vitro Potent Antiviral Activities of Dextran Sulfate and Pentosan Sulfate against Viruses in the HIV Family

When susceptible interleukin 2 (IL-2)dependent helper T-cells, ATH8 cells, were exposed to HIV-1 in the form of cell-free virions, essentially all ATH8 cells were destroyed by the cytopathic effect of HIV-1 by day 7 in culture. However, when ATH8 cells were cultured in the presence of more than 1.25 uM dextran sulfate, the target ATH8 cells were completely protected against the cytopathic effect of the virus and the cells could grow comparably to the virus-free control ATH8 population. Dextran sulfate can have two sulfate groups per glucose unit and these sulfate groups appear to be associated with its antiviral activity. Indeed, non-sulfated dextran with a molecular weight of 9,400 showed no protective effect on ATH8 cells against the virus even at very high concentrations. Dextran sulfate also protected normal helper/inducer clonal T-cells (TM11 cells) against the cytopathic effect of the virus. In this setting, 0.625 uM of this compound gave complete protection and at concentrations up to 12.5 uM the growth of the T cells was not suppressed. We also found that dextran sulfate at comparable concentrations inhibits the *in vitro* infectivity and cytopathic effect of human immunodeficiency virus type 2 (HIV-2).

We tested another anionic polysaccharide, pentosan sulfate *in vitro*. Pentosan sulfate exerted a potent anti-HIV activity in the cytopathic effect inhibition

assay using ATH8 cells as target cells. Pentosan sulfate was as effective as dextran sulfate on the basis of molarity.

As an additional index of anti-retroviral effect we asked whether viral DNA could be detected in susceptible ATH8 cells exposed to HIV-1 but protected by dextran sulfate. In the absence of the drug, viral DNA was first detected on day 2, and on day 4, a substantial amount of viral DNA was detected. In contrast, in T-cells similarly exposed to the virus and cultured in the presence of 2.5  $\mu$ M dextran sulfate, neither unintegrated viral DNA nor integrated proviral DNA was detected throughout the study, suggesting that dextran sulfate inhibits HIV-1 at the early step(s) of infection.

#### Inhibition of HIV Reverse Transcriptase Is Unlikely to be an Antiviral Mechanism of Dextran Sulfate

To investigate the mechanism of anti-HIV effect of dextran sulfate, we asked if dextran sulfate could inhibit the viral DNA polymerase (reverse transcriptase) activity, using a synthetic template. Dextran sulfate showed a potent inhibitory activity against purified HIV-1 reverse transcriptase at  $\geq 1$   $\mu$ M and the reverse transcriptase mediated DNA synthesis was virtually completely inhibited. This compound at comparable concentrations, however, also suppressed the DNA synthesis mediated by mammalian DNA polymerase alpha, an enzyme that has key DNA synthetic and repair functions. The 50% inhibitory dose ( $ID_{50}$ ) of dextran sulfate against either DNA polymerase was found between 0.125  $\mu$ M and 1.25  $\mu$ M. Furthermore, the addition of 50  $\mu$ g/ml and 100  $\mu$ g/ml of non-enzyme protein, bovine serum albumin, readily nullified the reverse transcriptase inhibition by 1.25  $\mu$ M dextran sulfate from 99% to 47% and 14%, respectively. In light of these results, the capacity of dextran sulfate to block the infectivity of HIV without toxicity might be rather difficult to attribute to selective inactivation of viral reverse transcriptase *per se*. However, such an effect cannot be excluded.

#### Inhibition of Syncytia Formation by Dextran Sulfate

Fusion events are thought to be mediated by sequences in the gp 41 portion of the envelope. The integrity of tertiary complexes of viral envelope protein is likely to be crucial for these processes. A drug such as dextran sulfate might function at any of these points. To determine if dextran sulfate could inhibit the syncytia formation, we employed a system of binding of CD4<sup>+</sup> uninfected cells to viral protein expressed on the surface of HIV-1-infected cells for the induction of giant cell formation. When CD4<sup>+</sup> ATH8 cells were co-cultured with HIV-1-infected H9 cells, substantial numbers of syncytia (giant cells) were formed in 48 hr. The continuous presence of 3'-azido-2',3'-dideoxythymidine (AZT) and 2',3'-dideoxyadenosine, viral DNA-chain terminators known to be effective against HIV-1 replication at the stage of reverse transcription, failed to inhibit the syncytia formation. Dextran sulfate at 6.75  $\mu$ M, however, completely inhibited syncytia formation between ATH8 cells and HIV-1-infected H9 cells.

#### CD4 Expression is not Affected by Dextran Sulfate

Fusion of uninfected CD4<sup>+</sup> cells with HIV-infected cells can be blocked by certain anti-CD4 antibodies. In the absence of such antibodies, the syncytia produced



die shortly after formation and surviving population can be comprised of resistant CD4 negative cells. To ask if dextran sulfate modulated or down-regulated CD4 antigen expressed on the surface of the target T-cells to yield HIV-resistant T-cells, we determined the quantity of CD4 molecule detected by OKT4A antibody, expressed on the normal helper T-cells (TM11), susceptible helper T-cells (ATH8), and relatively permissive cells (H9), by an immunofluorescence technique. We found no significant change in the CD4 immunofluorescence intensities in any of these target Tcells after exposure to dextran sulfate.

#### Inhibition of HIV Binding by Anionic Sulfate and Pentosan

We then asked if two anionic polysaccharides could block the binding of HIV-1 to CD4<sup>+</sup> T-cells. Tritiated-uridine-labelled HIV-1 virions derived from the molecular clone, HX10, were incubated with uninfected H9 cells in the presence or absence of various concentrations of the drugs, and the radioactivity of virions bound to the cells was determined. By analysing the bound radioactivity on a per-cell basis, we generally found 500 to 2,000 virus particles bound to a cell in the absence of drug under the conditions used. Comparable data have been obtained when different cell lines including Jurkat cells and Molt-3 cells or a different molecularly cloned virus strain, WMJ-1, were used. To express the level of virion binding inhibition displayed by various compounds, the specific inhibition was calculated such that the amount of labelled virions bound in the presence of a supermaximal concentration of OKT4A (50 ug/ml) represented 100% specific inhibition, and the amount bound in the absence of drug or antibody represented 0% specific inhibition. At comparable concentrations of anti-CD8 antibody (OKT8) virtually no inhibitory effect on viral binding was seen. Dextran sulfate exhibited an essentially complete inhibitory effect on the binding of radio-labelled virus to H9 cells. Level of binding inhibition by dextran sulfate was equal to the maximal inhibition achieved by the OKT4A monoclonal antibody at as low as 1 uM. Non-sulfated dextran, which is inert against HIV-1, showed no inhibition. Pentosan sulfate also blocked the binding of HIV virions to CD4<sup>+</sup> T cells at similar concentrations as compared to dextran sulfate.

#### DISCUSSION

At least two viral structural determinants are involved in the process of HIV penetration into a target cell: the CD4 binding site within gp120 and a fusogenic domain believed to reside within the transmembrane env protein of HIV, gp41. In this respect, certain antibodies may be capable of inhibiting effective viral binding by blocking the CD4-attaching site of gp120 and/or blocking access to the fusogenic domain. Our current data suggest that two anionic polysaccharides, dextran sulfate and pentosan sulfate, are also capable of inhibiting virion attachment, although other mechanisms (e.g. interference with retroviral uncoating or interference with reverse transcriptase compartmentalized to the cytoplasm) could be involved in its anti-HIV effect. The precise way in which dextran sulfate and pentosan sulfate affect virion binding is not established and will require further research.

It might be possible to get more than additive antiviral effects and thereby lessen side effects of therapy if multiple drugs having different antiviral mechanisms are properly combined. Indeed, we have observed that combinations of

dextran sulfate and certain DNA chain-terminating dideoxynucleosides including AZT and 2',3'-dideoxycytidine work against the virus better than each drug does alone in vitro (Mitsuya et al, unpublished). The phase I/phase II clinical trial has been started and preliminary data have shown that dextran sulfate is tolerated in certain patients with AIDS and ARC. While it is premature to assess clinical results, it is conceivable that therapies that alter virion binding will alter the pathogenesis of HIV infection. A definitive answer and further drug development will depend on carefully performed controlled clinical trial.

#### PUBLICATIONS

1. Mitsuya H, Looney DJ, Kuno S, Ryuji U, Wong-Staal F, Broder S. Inhibition of virion binding to CD4<sup>+</sup> cells: suppression of human immunodeficiency viruses by anionic polysaccharides. In: Groopman J, Evan C, Golde D, eds. Mechanisms of action and therapeutic application of biologicals in cancer and immune deficiency syndrome. New York: Alan R. Liss, 1989; in press.
2. Mitsuya H, Hayashi S, Yarchoan R, Aoki S, Currens MJ, Matsukura M, Broder S. Strategy of targeted antiretroviral therapy against human immunodeficiency virus (HIV). In: Groopman JE, Chen I, Essex M, Weiss R, eds. Human retroviruses. UCLA Symposia on Molecular and Cellular Biology, New Series, Volume 119. New York: Alan R. Liss, 1989, in press.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 CM 07202-06 BDMS
PERIOD COVERED <u>October 1, 1988 through September 30, 1989</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Biostatistics and Data Management Section</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Seth M. Steinberg	Acting Head BDMS, COP, DCT, NCI
Other:	David J. Venzon	Senior Investigator BDMS, COP, DCT, NCI
COOPERATING UNITS (if any)		
LAB/BRANCH		
SECTION <u>Biostatistics and Data Management Section</u>		
INSTITUTE AND LOCATION <u>NCI, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>             The Section is the statistical and data management component of the Clinical Oncology Program (COP). The Section provides statistical leadership and data management consultation for major activities of the Program, and is involved in the design, conduct, monitoring, and statistical analyses of intramural and national multicenter clinical trials of experimental treatments for cancer. Other major collaborative efforts include studies to identify important prognostic and treatment selection factors, evaluate diagnostic procedures, develop improved staging systems, and assist investigators in the design, execution, and analyses of major in vitro drug testing studies. The Section develops new statistical designs and biometric methods related to the development and evaluation of new cancer treatments. The Section maintains computerized data collection systems for intramural and national multicenter clinical protocols, and it works closely with interested branches to improve data recording and retrieval. The Section is working to develop specialized clinical data bases for individual branches within the COP. The Section works with the Clinical Center Medical Information System team, allowing COP input into decisions which directly impact patient care and protocol management. The Section assists the Deputy Clinical Director to insure adequate monitoring of protocols through the MIS Toxicity screens and other mechanisms.           </p>		

# 1. Collaborative Projects Within Clinical Oncology Program

Members of the Biostatistics and Data Management Section provide to the intramural clinical research program both biostatistical and data management expertise. Our efforts in these areas are described in Sections A) and B) below.

A. Members of the Biostatistics and Data Management Section (BDMS) participate in the development of new protocols and the interim monitoring and data collection for ongoing studies. A member of the Section also serves on the Clinical Research Sub-Panel to review all intramural clinical trials. BDMS staff collaborate in clinical and laboratory studies to evaluate prognostic and treatment selection factors and elucidate tumor biology. The Section provides statistical support for the COP as well as advice on the best ways to use available NIH computer systems or microprocessor based professional workstations for clinical and laboratory research. The Section is also presently developing extensive microcomputer based data management systems for several branches in the COP.

A detailed list of COP projects to which members of the Section have provided statistical input follows:

- (1) Performed two interim analyses of ALL (leukemia) protocol 77-02, a cooperative study with 181 patients at five institutions.
- (2) Performed two interim analyses of two ALL protocols for average and high risk patients; the high risk protocol is a single arm extension of the successful chemotherapy only (no cranial irradiation) arm on the multi-institutional 77-02 protocol, with modifications in Ara-C administration to prevent CNS relapse and to more aggressively treat systemic disease. The average risk protocol is a randomized extension of 77-02, comparing two chemotherapy only arms -- one with high dose methotrexate and one without.
- (3) Performed interim analysis of four phase II trials of 6MP in 85 patients at over a dozen institutions participating in the Pediatric Oncology Group.
- (4) Arranged for randomizations and eligibility checklists for protocols to be conducted through Surgery Branch, Medicine Branch and Pediatric Branch.
- (5) Served as member on Institutional Review Board.
- (6) Performed major update of results on all soft tissue sarcoma protocols, comparing adjuvant chemotherapy to no chemotherapy in patients with extremity tumors, and with head, neck and trunk tumors, comparing limb-sparing surgery to amputation in patients receiving adjuvant chemotherapy, comparing a short course adjuvant chemotherapy regimen (350 mg/m<sup>2</sup> doxorubicin) with standard course (550 mg/m<sup>2</sup>); and comparing radiation to no radiation in patients with high grade soft tissue sarcoma of extremities with local surgical resection or with low grade soft tissue sarcoma of head, neck and trunk, or extremities.
- (7) Performed analyses to determine relationships between food intake, weight, and tumor size on growth, cachexia, and survival in rats receiving TNF injections.
- (8) Analyzed data relating to prognostic factors in long term survival on patients with Ewing's sarcoma.
- (9) Performed analyses of data on multidrug resistance in transfected rat cell lines.

- (10) Performed analysis of data on survival of patients undergoing pulmonary metastatectomy for renal cell cancer.
- (11) Provided advice to a senior investigator regarding interpretation of statistical analyses appearing in a manuscript for which he was a referee.
- (12) Prepared statistical considerations for a replacement randomized protocol for patients with stage I or II breast cancer.
- (13) Reviewed a manuscript for the Journal of the National Cancer Institute, at the request of its editor-in-chief.
- (14) Performed analyses of data regarding prediction of outcome in patients with cancer who develop fever while undergoing therapy.
- (15) Analyzed dose intensity of drugs delivered in relation to outcome in patients with lymphomas treated on a Pediatric Branch protocol.
- (16) Prepared revisions to a manuscript on stimulation of bone resorption by conditioned media in a prostate cancer cell line.
- (17) Analyzed data on several measurements of bactericidal activity of neutrophils in HIV patients and normal controls.
- (18) Performed analyses of data on the effects of laminin on the action of multiple drugs in several cell lines.
- (19) Performed analyses on the effects of TNF on the action of two drugs in several cell lines.
- (20) Performed preliminary analyses of cytophotometry and flow cytometry data on patients with the MFH histology of soft tissue sarcomas treated at the NCI.
- (21) Analyzed data on the effects of IUdR on brain tumor patients.
- (22) Performed analyses of data demonstrating effects of rehabilitation in women treated on the randomized early breast cancer trial.
- (23) Performed analyses of data from the randomized trial of aggressive vs. conservative therapy for treatment of mycosis fungoides.
- (24) Analyzed the effects of changes in staging technology on prognostic factors in small cell lung cancer.
- (25) Performed analyses of data for a study of Ewing's sarcoma in children and young adults.
- (26) Performed analyses of T4 counts on patients with AIDS treated on an AZT protocol.
- (27) Provided advice regarding design of a phase I-II study of M-CSF in patients with advanced cancer.
- (28) Performed updated analyses of three protocols for treatment of osteosarcoma conducted by Surgery Branch.
- (29) Analyzed data from a protocol for treatment of extensive stage small cell lung cancer.
- (30) Developed statistical considerations for a phase II study of combination chemotherapy + GM-CSF for treatment of stage III/IV breast cancer.
- (31) Participated in several end-of-year protocol review sessions conducted by Surgery Branch.
- (32) Prepared statistical considerations for a randomized trial of GM-CSF in pediatric patients.
- (33) Performed analyses of data used to predict whether a pheochromocytoma is benign or malignant.
- (34) Performed analyses of data on cardiotoxicity of doxorubicin in patients on ongoing clinical trials.
- (35) Analyzed data on the effect of TNF on the survival of several cell lines in the presence of doxorubicin and etoposide.

- (36) Performed analysis of risk factors for patients undergoing chest wall resection for sarcomas.
- (37) Performed analyses of data relating genotypic and cytogenetic data to clinical data and outcomes in children with ALL.
- (38) Performed analyses of data from the locally advanced breast cancer protocol.
- (39) Prepared statistical considerations for a randomized comparison of three different forms of administration of AZT to children with AIDS.
- (40) Performed analyses of effects of blood transfusions on patients in the randomized advanced cancer protocol comparing IL-2 to IL-2+LAK.
- (41) Performed analyses of results from a single-arm study of IL-2 + alpha-interferon for treatment of patients with advanced cancer.
- (42) Prepared statistical considerations for a phase II study of IL-2 + RT + TILs in patients with advanced cancer.
- (43) Determined that it was not appropriate to stop accrual to a study of multi-modality therapy in limited non-small cell lung cancer patients.
- (44) Provided advice regarding design of a pilot study comparing peripheral blood drawing vs. central line drawing for measuring PT and PTT in a trial of Suramin.
- (45) Provided advice regarding design of a trial of a salvage regimen for patients with renal cancer who failed combination therapy.
- (46) Provided advice regarding randomization for psychological as well as medical factors in clinical trials for cancer and AIDS.
- (47) Provided advice regarding design of a long course AZT therapy trial for patients with AIDS.
- (48) Performed analysis of data from a prospective clinical trial of individualized chemotherapy and prediction of chemotherapeutic response based on in-vitro drug sensitivity testing in extensive stage small cell lung cancer.
- (49) Performed analyses of data resulting from a study of multiple thoracotomies in patients with soft tissue sarcomas.
- (50) Analyzed several measures of polymorphonucleocytic activity in HIV-positive patients and tested additional data for activity, in the presence of various drugs, of the PMN's of normal controls.
- (51) Performed analyses of differences between cell survival curves in serum-supplemented and serum-free media.
- (52) Reviewed the statistical aspects of a pilot study of interferon gamma and MTP-PE.
- (53) Performed survival analyses of data on a study of malignant brain tumors.
- (54) Proposed statistical considerations for a protocol of Ara-AC in breast cancer.
- (55) Prepared statistical considerations for a protocol comparing placebo to somatostatin analog with respect to effectiveness in reducing drainage following surgery for islet cell tumor.
- (56) Performed analysis of data on patients with fever randomized to amphotera-cin vs. no amphotera-cin.
- (57) Provided advice regarding study of escalating doses of a combination of drugs in a variety of patients with cancer.
- (58) Provides advice regarding comparison of treatment vs. control in experiments involving feeding of rats over several days.
- (59) Performed analyses of data from a study of the use of bombesin and related immunohistochemical substances to predict clinical outcome of patients

with lung cancer.

- (60) Performed analyses regarding the relationship between dose intensity and outcome on the locally advanced breast cancer study.
- (61) Performed analyses comparing fault sites in normal volunteers vs. patients with lung cancer vs. smokers.
- (62) Analyzed data comparing drug IC50 levels in a parent cell line with nine transfected descendant lines.
- (63) Performed annual updated analysis of randomized early breast cancer trial.
- (64) Prepared statistical considerations for a phase II trial of Suramin for metastatic prostate cancer.
- (65) Performed analyses of data on chest wall resection patients.
- (66) Provided advice regarding sample sizes needed for experiments on rescue from toxicity of chemotherapeutic agents.
- (67) Provided advice and analyses to address whether to terminate accrual to the randomized trial of surgery alone vs. surgery + IL-2 + LAK for patients with a colorectal primary and liver resection.
- (68) Performed analyses of T4 count changes in patients with AIDS treated with ddC.
- (69) Performed analyses for a study of liver function during IL-2 administration.
- (70) Prepared statistical section for phase II trial of combination chemotherapy for breast cancer.
- (71) Analyzed data for relation between activity and drug sensitivity of 24 cell lines, and for comparison of survival curves in serum-free and serum-supplemented media.
- (72) Prepared statistical considerations section of a pilot protocol for use of GM-CSF in the treatment of small non-cleaved and diffuse large cell lymphomas.
- (73) Provided advice regarding data on photosensitizer update normalized by cell size and protein content.
- (74) Analyzed data on cell linking activity of GM-CSF activated cells.
- (75) Prepared statistical considerations for a prospective study of atrial natriuretic factor in patients with small cell lung cancer.
- (76) Performed analyses of data from a study of platinum DNA adjunct levels in patients with ovarian cancer.
- (77) Analyzed correlations between outcomes of febrile episodes and microbiology data in patients with aplastic anemia.
- (78) Developed and applied a statistical method for comparing coefficients of variation in HPD concentrations. Analyzed experimental data on differences in HPD over time and site.
- (79) Analyzed data on complications in patients randomized to Hickman or Port-a-cath catheters.
- (80) Provided written discussion of statistical power considerations in the extensive small cell lung cancer protocol.
- (81) Performed analyses of data for a study predicting outcome in patients with fever treated with ceftazadime.
- (82) Performed analyses of clinical trials involving IL-2.
- (83) Provided statistical considerations for a phase II study of thiotepa in pediatric CNS tumors.
- (84) Performed analyses of quality of life data in patients with soft tissue sarcoma.
- (85) Performed analyses for a study of expression and amplification of genes

in primary breast cancer.

(86) Performed analyses of data for a study of the relationship of ceruloplasmin and outcome in patients with locally advanced breast cancer.

(87) Provided advice regarding dose escalation in radiation therapy protocols.

(88) Provided sample size considerations in an experiment on in-vitro calcium release due to parathyroid hormone.

## B. Data Management Activities

The Section has continued the development and maintenance of several systems which facilitate the monitoring of protocols:

(1) CAPRI II, an updated DB2 version of the current CAPRI system, is being designed, developed, programmed and documented. The system will include a set of core data fields which will be required for all patients, as well as existing data elements available in the current CAPRI system. The system will have the capability to upload data from the PC data management systems of each branch to update the DB2 database.

(2) The CAPRI system, the primary COP database, has been continuously updated, and upon completion of CAPRI II all existing data will then reside in the new system.

(3) Continued to provide data management support for the Surgery Branch through system enhancement and initiation/completion of ten data entry forms used in the Surgical Oncology Lymphokine Immunotherapy Data (SOLID) system, maintained on the IBM-370 mainframe with WYLBUR and SAS.

(4) Development and programming continued on the Protocol Database Management System (PDMS) in 4th Dimension on the Macintosh for the Medicine Branch and the Pediatric Data Management System in dBASE III+ for the Pediatric Branch.

(5) An analysis was performed for the Radiation Branch to determine the software for the Branch Data Base Management System. Foxbase+/MAC was chosen and a preliminary system was designed. Additional analyses continue with the focus being on adopting the Pediatric Data Management System and adapting the system for the Radiation Branch.

(6) A variety of graphs, plots and reports have been provided to COP branches.

(7) In support of the COP use of personal computers, assistance and consultation was provided in the selection and installation of hardware, evaluation of software packages, and the design and implementation of scientific PC programs.

A detailed list of data management projects undertaken by members of the BDMS for the COP follows:

(1) Collection, maintenance, and reporting of basic survival and protocol entry data on COP active patients.

(2) Data management, programming, retrievals, analyses and reports as required by all branches of the COP.

(3) Placement of data managers in the Medicine, Pediatric, and Radiation Oncology Branches, to reach the goal of having data managers in each branch of the COP.

(4) Development and revision of data collection forms, as required, for all branches of the COP.

(5) Create, modify and update databases for researchers of the COP.



- (6) Support to insure that all patients receiving chemotherapy, especially investigational drugs, have a valid Clinical Center protocol number for pharmacy records.
- (7) Maintenance of various computer packages and hardware used by the Section.
- (8) Maintenance of a computer file of the latest actual outpatient clinic visit for all active patients.
- (9) Provide computer needs analysis and evaluation, and equipment and software purchase recommendations for acquiring personal computers.
- (10) Assist research nurses, principal investigators, and clinical associates in the training and use of personal computers for protocol data management.
- (11) Maintenance of major statistical and plotting programs for PCs to allow BDMS statisticians to perform Kaplan-Meier curves, logrank tests (including stratified version), logistic regression, and Cox regression.
- (12) Collaboration with Clinical Center computer staff on abstracting and downloading MIS data for protocol uses.
- (13) Automatic printing of all data from the MIS Toxicity and Protocol Monitoring System as progress notes for review and monitoring, and distribution of these documents to the appropriate investigators.
- (14) Provided support to the Radiation Oncology Branch early stage breast cancer protocol including keying of data and preparation of programs and reports.
- (15) Continued support of COP randomization activities with the revision of several existing protocols and the addition of two new protocols.
- (16) Began registering all Medicine Branch patients entered on any protocol, including the creation and completion of eligibility checklists and logs for 46 protocols.
- (17) Acted as a coordinating center for three multi-center pediatric leukemia protocols, involving registration of patients, data collection, processing, analysis and reporting.
- (18) Served as a coordinating center for two multi-center ovarian cancer protocols, including data collection and maintenance of protocol databases.
- (19) Completed modifications to the patient tracking systems for the Pediatric and NCI-Navy Medical Oncology Branches.
- (20) Designed, developed, programmed, implemented, and installed the NCI-Navy Medical Oncology Branch pathology tracking system written in dBASE III+.
- (21) Completed the development and programming of the Metabolism Branch data management system using Foxbase+ on the IBM PC, including upgrades and enhancements, and are currently making final arrangements for the installation of a graphics package.
- (22) Continued development and programming of the Medicine Branch protocol database management system using 4th Dimension on the Apple Macintosh. New protocol databases are being added to the system and modifications and enhancements are being made as needed. Programs were completed for the tape transfer of protocol and toxicity data from the Medicine Branch to CTEP. Modifications are made to the process as required. Documentation on the system continues, including the system, maintenance, and user documentation.
- (23) Programming, testing, and documentation continued on the Pediatric Branch data management system using dBASE III+ on the IBM PC. Data has been added to the patient information and protocol status areas. Research was performed on how to facilitate the transfer of laboratory data from the MIS and CIU. The system is also currently being converted to Foxbase+/Mac, so that it may

be used on the Macintosh.

(24) A data manager was placed in the Radiation Oncology Branch and is responsible for collecting data on all Branch patients and adding them to the new data management system using Foxbase+/Mac on the Macintosh.

(25) Two data managers were added to the Medicine Branch to assist research nurses in data collection, processing and management. The data managers are responsible for collecting and keying data into the new system, monitoring and reviewing protocol databases, forms design, running tapes for CTEP and providing general data management support as needed.

(26) Two data managers were added to the Pediatric Branch staff. The data managers are providing data management support, including review of charts, forms completion, database updating and reviewing, and overall support as requested.

(27) A data manager has been entering data for the Cytogenetics Laboratory of the Medicine Branch.

(28) Psychological data from questionnaires completed at Camp Fantastic during the past five years was keyed and edited.

## 2. Projects Outside COP

A. The BDMS also participates in biometric activities outside of the COP. A detailed list of projects outside of COP in which the Section's statisticians have provided statistical input include the following:

(1) Prepared chapter on statistical considerations for clinical trials for NIH Clinical Center manual, "How to Write a Protocol".

(2) Prepared statistical considerations and randomization procedures for randomized trial, to be conducted by NIDDK, of radioiodine + adriamycin for the treatment of follicular thyroid cancer.

(3) Served in a continuing capacity as statistical advisor to the Scientific Director of the National Center for Nursing Research.

(4) Prepared material for presentation at a Clinical Center Grand Rounds.

(5) Performed analysis of data on Hodgkins' lymphoma for a pathologist on the staff of the Laboratory of Pathology, Clinical Center.

(6) Reviewed a manuscript for a physician in the Diagnostic Radiology Department, Clinical Center.

(7) Provided advice to a senior investigator in the NCI Biological Response Modifiers Program (BRMP) regarding design of three parallel phase II study arms within one trial.

(8) Advised a senior investigator in the NCI BRMP regarding presentation of laboratory data collected during a clinical trial.

(9) Advised a senior investigator in the NCI BRMP regarding analysis of IL-2 catheter complications data.

(10) Advised a senior investigator in the NCI BRMP regarding design and analysis of a phase I trial of anti-CD3 antibodies.

(11) Provided advice to a senior investigator in the NCI BRMP on analyses regarding comparison of complication rates of antibodies vs. no antibodies in catheters.

(12) Advised the Chief of the Clinical Research Branch, BRMP regarding statistical considerations for a phase I trial of GM-CSF in patients with cancer restricted to the peritoneal cavity.

- (13) Analyzed alternative models of inhibition of drug uptake in multidrug-resistant cells for a visiting scientist in Laboratory of Molecular Biology (LMB), DCBD.
- (14) Analyzed MDRI RNA levels in treated and untreated samples for a senior investigator in LMB, DCBD.
- (15) Provided advice to the Dean of the National Cancer Institute of Egypt regarding the ways in which the U.S. NCI is providing data management and statistical support for its intramural clinical trials.
- (16) Prepared statistical considerations for a protocol testing trimetrexate as a treatment for psoriasis for a senior investigator in Dermatology Branch, DCBD.
- (17) Analyzed data on chimerism in lethally irradiated rats after bone marrow injections depleted by different monoclonal antibodies for an investigator in the Immunology Branch, DCBD.

B. In addition to data management support for intramural trials, the BDMS provides data management services outside the COP. Project staff have provided operations and/or statistical center support to a number of multi-institutional extramural trials. This support includes performing randomizations, design of data collection instruments, software design and development, production of regular status reports, and production of ad hoc reports and tabulations as directed by the study statistician. The extramural trials supported include:

- (1) 7601/7602, Treatment of Early Stage Ovarian Cancer
- (2) CCSG-191P, CCSG Protocol for Acute Lymphoblastic Leukemia
- (3) CCSG-134P, CCSG Protocol for Poor Prognosis Acute Lymphoblastic Leukemia
- (4) CCSG-144P, CCSG Protocol for Average Prognosis Acute Lymphoblastic Leukemia

### 3. Biometric Research

Current biostatistical research being conducted includes:

- (1) A two stage method for selected interactions between variables to be evaluated for prognostic importance.
- (2) Development of data management systems which may serve multiple purposes.
- (3) Development of models for testing in-vitro synergy of chemotherapeutic agents.
- (4) Extensions and applications for models of correlated binary observations.
- (5) Development of estimation methods for parametric transformations in survival analyses.

### Publications:

- 1. Glenn J, Kurtzman SH, Steinberg WM, Steinberg SM, Sindelar WF. Evaluation of the utility of a radioimmunoassay for serum CA 19-9 levels in patients with carcinoma of the pancreas. J Clin Oncol 1988;6:462-8.
- 2. Lefor AT, Steinberg SM, Wiebke EA. Analysis of 51-chromium release assay data using personal computer spreadsheet software. Comput Biomed Res 1988; 21:268-75.

3. Manyak MJ, Smith PD, Harrington FS, Steinberg SM, Glatstein E, Russo A. Protection against dihematoporphyrin ether photosensitivity. *Photochem Photobiol* 1988;47:823-30.
4. Lefor AT, Merino M, Steinberg SM, Dwyer A, Roth JA, Flanagan M, Pass HI. Computerized tomographic prediction of extraluminal spread and prognostic implications of lesion width in esophageal carcinoma. *Cancer* 1988;62:1287-92.
5. Swain SM, Steinberg SM, Lippman ME. Salvage treatment with intermediate dose Methotrexate and 5-Fluorouracil in metastatic breast cancer. *Am J Clin Oncol* 1988;11:445-7.
6. Medberry CA, Strauss KL, Steinberg SM, Cotelingham JP, Fisher WS. Low-grade astrocytomas: Treatment results and prognostic variables. *Int J Rad Oncol Biol Physics* 1988;15:837-41.
7. Swain SM, Steinberg SM, Bagley C, Lippman ME. Tamoxifen and Fluoxymesterone Tamoxifen and Danazol in metastatic breast cancer - A randomized study. *Breast Cancer Res Treat* 1988;12:51-7.
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## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06119-20 M

PERIOD COVERED		
October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders ) Cytogenetic studies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator ) (Name, title, laboratory, and institute affiliation)		
Jacqueline Whang-Peng, M.D.	Principal Investigator	MB, COP, DCT, NCI
Turid Knutsen	Medical Technologist	MB, COP, DCT, NCI
Elaine Lee	Medical Technologist	MB, COP, DCT, NCI
Wing-Keung Chau	Guest Researcher	MB, COP, DCT, NCI
COOPERATING UNITS (if any) Environmental Epidemiology Branch, NCI; Medial Oncology Branch, NCI/Navy; Surgery Branch, NCI; Pediatric Oncology Branch, NCI; Laboratory of Tumor Virus Biology, NCI		
LAB/BRANCH Medicine Branch		
SECTION Cytogenetic Oncology Section		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 4.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>The Cytogenetic Oncology Section has been examining specimens and tissue culture lines established from patients with hematologic malignancies and solid tumors in order to identify specific chromosomal changes associated with or diagnostic of these diseases. We are investigating the contribution of inherited chromosome abnormalities and the susceptibility of peripheral lymphocytes to breakage (fragile sites) to the development of these diseases. As part of the investigation of the etiology of the fragile sites, we are studying the relationship between topoisomerase II and the expression of fragile sites. In addition, the laboratory is using chromosomal in situ hybridization to localize multiple drug resistance genes, to identify viral integration sites, and to localize other genes that may be important in the development of malignant diseases</p>		

1. Cytogenetic studies of human neoplastic hematologic, and congenital diseases, with special emphasis on patients with AIDS who develop leukemia, lymphoma, or Kaposi's sarcoma. Specific diseases studied include lymphoma (Burkitt's and non-Burkitt's), non-small cell carcinoma of the lung, rhabdomyosarcoma, renal cell carcinoma, small-cell tumors in childhood, acute lymphocytic leukemia, preleukemia, secondary leukemia, and esophageal cancer.
2. Cytogenetic studies in rhabdomyosarcoma; direct studies of specimens from 12 patients and studies of 8 established cell lines.
3. Localization of genes in normal chromosomes, using in situ hybridization.
4. Cytogenetic and viral integration studies (using in situ hybridization) of human foreskin cell lines infected with human papilloma virus.
5. Fragile sites studies (peripheral blood) in patients with:
  - a. Small cell lung cancer (SCLC)
  - b. Non-small cell lung cancer
  - c. Cyclic neutropenia
6. Cytogenetic studies of cell lines infected with HIV-1.
7. Study of the relationship between topo II and fragile sites. Human lymphocyte chromosomes are labeled with  $^3\text{H}$  thymidine, and then subjected to the fragile site harvesting procedure. The cleavable complexes are trapped with SDS and the protein; DNA complexes analyzed to determine if topo II is the protein involved in these complexes. In the original experiment, the amount of trapped protein was too small to detect the presence of topo II in the DNA complex. It has been proposed that the fragile site is a nucleosome free region. In collaboration with Dr. Berton Zbar, Ruth Neta, and Julang Huang, the working probes D3S3 and D3S2 (polymorphic alleles to Msp1) will be used.

#### Projects Completed

1. Completion and publication of cytogenetic review chapters on:
  - a. Polycythemia vera.
  - b. Neoplastic diseases in humans and animals.
  - c. Non-Hodgkin's lymphoma.
  - d. Double minutes, HSRs, and drug resistance.
2. Chromosomal localization of the following genes:
  - a. Human anionic glutathione S-transferase cDNA at 11q13.
  - b. Human glutathione S-transferase Ha gene at 6p12.



3. Loss of heterozygosity of the retinoblastoma gene (Rb) in lung cancer . A study of 8 primary SCLC tumor and 50 cell lines from all types of lung cancer revealed structural abnormalities of the Rb gene (located at 13q14) in SCLC and pulmonary carcinoids (both neuroendocrine tumors) but not in other major types of lung cancers. These results suggest that this gene may be involved in the pathogenesis of neuroendocrine lung cancer.

4. Cytogenetic studies of esophageal carcinoma:

All 14 cell lines (both short- and long-term lines) showed extensive numerical and structural abnormalities involving every chromosome including the sex chromosomes, indicating that these abnormalities occur early in the malignant cells. The chromosomes most frequently involved in structural changes in 1, 3, 9, and 11; the most frequent breakpoints occurred at 3p14, 11q11q12, and 9q11q12, and the centrometric regions off all of the acrocentric chromosomes. An HSR at 11q12 was found in three lines.

5. Molecular and cytogenetic studies of 5 patients with extra pulmonary small cell cancer showed that chromosome 3p is typically retained in this group of tumors.
6. Cytogenetic studies of cell lines from 30 patients with N-SCLC (in preparation). This project included studies of the following types of N-SCLC: squamous cell carcinoma; adenocarcinoma; bronchioalveolar carcinoma, large cell carcinoma; adenosquamous; carcinoid tumors; mucoepidermoid tumors; and mesothelioma. Almost all cases showed a wide distribution of chromosome numbers, with multiple modes. All chromosomes were involved in numerical and structural abnormalities. Chromosomes 3, 1, 7, 17, 11, and 12 were the most frequently involved in marker formation (listed in decreasing order) and the most highly involved sites were 3p14.2, 3p21, 1q32, 11p15, 1q21, 3p21, 7q21, 19q13, and 15p11. Deletion of 3p14p23, seen in SCLC, was observed in 14 of the 30 cases: squamous cell (1/4 cases), adenocarcinoma (3/7), bronchioalveolar (3/3), atypical carcinoid (3/3), and mesothelioma (2/2).

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## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06513 13 M

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology of Antimetabolite Agents

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Bruce A. Chabner, M.D.

Director, DCT

OD, DCT, NCI

Carmen Allegra, M.D.

Principal Investigator

MB, COP, DCT, NCI

Keisuke Aiba, M.D.

Guest Researcher

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Patrick Elwood, M.D.

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Clement Knight, M.D.

Visiting Fellow

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Donna Boarman

Biologist

MB, COP, DCT, NCI

James C. Drake

Biologist

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Sydell Zinn

Biologist

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Edward Chu, M.D.

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Patrick Johnston, M.D.

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## COOPERATING UNITS (# any)

NCI-Navy Oncology Branch, COP, DCT, NCI

Critical Care Medicine Department, Clinical Center, NIH

NCI Pediatric Oncology Branch, Clinical Center, NIH

## LAB/BRANCH

Medicine Branch

## SECTION

Gastrointestinal Tumor Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

## TOTAL MAN-YEARS:

5.5

## PROFESSIONAL:

4.0

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The section for the study of gastrointestinal tumors is divided into two broad areas that include the development of strategies for the treatment of gastrointestinal tumors and the development of therapies for the treatment of opportunistic infections in patients with AIDS. The antineoplastic investigations revolve around the development of a complete understanding of the mechanisms of action and the mechanisms of resistance to the antimetabolite class of antineoplastic agents, specifically, 5-fluorouracil and methotrexate. The central focus of this area is to improve the therapy of gastrointestinal cancer by 1) modulating the activity of the available antimetabolite agents in an effort to improve activity and circumvent resistance mechanisms defined from both preclinical and clinical investigations 2) enhancing the dose intensity of antimetabolite agents through use of biologic agents such as interferon and colony stimulating factors and 3) investigating the activity and mechanisms of action of novel agents for efficacy in the treatment of gastrointestinal malignancy. In addition, we are investigating the autocrine growth factor requirements for colorectal carcinoma and adenoma cells in an attempt to characterize and interfere with these growth requirements using specific agents such as suramin. By investigating the progression from adenoma to carcinoma we hope to understand how various autocrine factors may be involved in malignant transformation with the ultimate goal of preventing such transformation.

The investigations of therapies for opportunistic infections is focused on the interactions of antifolate agents on the metabolic pathways in *toxoplasma gondii* and *pneumocystis carinii*. In addition to the use of basic biochemical technologies, we are using the tools of molecular biology to provide quantities of the critical enzymes for characterization and to aide in the search for new therapeutic agents.

During Fiscal Year 1989, our work continued on the interactions of metabolites with mammalian and protozoan cells. Our major projects were the following:

1. Continued work on the mechanism of de novo purine and thymidylate synthesis inhibition by antifolates, including methotrexate, has strengthened a novel postulate that inhibition of metabolic pathways results from direct enzyme inhibition rather than via an indirect mechanism of folate depletion. These studies illustrate that de novo purine and thymidylate synthesis are inhibited by clinically relevant concentration of methotrexate without significant folate depletion in two human cell lines (breast MCF-7 and promyelocytic leukemia and myeloid progenitors from normal human volunteers).

These studies further suggest that the mechanism of metabolic inhibition is through direct inhibitory effects of accumulated dihydrofolate polyglutamates on the folate-requiring thymidylate synthase and AICAR transformylase. This work has been supported by corroborating the relative folate preservation during antifolate exposures using a highly sensitive and specific assay for intracellular folates. This assay was developed by Dr. Priest and is capable of directly measuring folates without the need for radiolabels with their attendant deficiencies.

2. During the course of the above studies, a novel physiologic folate was discovered, 10-formyl-dihydrofolate. Continued investigations have illustrated the formation of this compound in breast cells and in normal human myeloid progenitor cells. Identification and characterization of the enzyme responsible for the formation of dihydrofolate has been accomplished and the effects of this folate on folate-requiring enzymes has been investigated. The new folate is a potent inhibitor of thymidylate and GAR transformylase but, is an excellent substrate for AICAR transformylase, further supporting the concept that folate depletion is only a minor factor in the mechanism of action of methotrexate.

Studies directed toward a more complete understanding of leucovorin rescue may improve the clinical application of high-dose methotrexate, a strategy capable of overcoming most known mechanisms by which neoplastic cells become resistant to methotrexate. Detailed investigations of this phenomenon have shown that concentrations of folates in vast excess of these found in untreated cells are required to affect cell rescue from methotrexate, suggesting competition at the level of folate-dependent enzymes between reduced folates and direct enzyme inhibitors such as methotrexate and dihydrofolate polyglutamates. Further, dihydrofolate reached plateau levels in cells and this event may be interpreted as evidence for competition between dihydrofolate and the antifolate for DHFR activity as an important occurrence in the process of leucovorin rescue. Additional studies using dihydrofolate as a rescue agent have clearly illustrated that this folate is critical to the process of rescue and that the competitive interactions of MTX and leucovorin may be explained by the competition of MTX and dihydrofolate at the site of dihydrofolate reductase. These studies have important implications for the design of new therapeutic strategies utilizing high-dose MTX.

3. Drug resistance to antimetabolite agents is a major limiting factor in the therapy of malignant diseases. We have investigated the interaction of leucovorin with 5-fluorouracil in multiple colon cell lines developed by Dr. Park at the NCI-Navy Medical Oncology Branch. We have found that leucovorin can markedly enhance the potency of 5-fluorouracil and 5-fluorodeoxyuridine in selected lines. Currently, we are investigating the molecular mechanism of this interaction and we are investigating the additional mechanisms of resistance in the setting of combination treatment with fluoropyrimidines and leucovorin. Parallel studies are ongoing using tumor samples from patients with breast cancer who are being treated with 5-fluorouracil and leucovorin. Studies with human colon cell lines show that 5-FU resistance in these cells may result from differences in the incorporation of 5-FU anabolites into RNA. The reason for these differences does not appear to be based on differences in activating enzyme levels and the precise explanation are presently under investigation. In a recently completed trial using 5-FU and leucovorin for the treatment of 54 patients with metastatic breast cancer, we noted a 25% overall response rate in heavily pretreated patients who (90%) failed prior therapy with 5-FU. Serial tissue samples obtained from 20 patients demonstrated a marked enhancement and persistence of thymidylate synthase binding by 5-FU in the presence of leucovorin compared to patients treated without leucovorin. Of interest, 5-FU was noted to produce an acute (24 hours) increase in thymidylate synthase expression (2.5-fold). The mechanisms of this event maybe important in drug resistance and are currently under investigation.

One of the major mechanisms of resistance identified thus far appears to be an ability of malignant cells to overexpress the target enzyme, thymidylate synthase upon exposure to 5-FU. We have found that this mechanism may be obviated by the simultaneous use of gamma-interferon which results in the reversal of 5-FU resistance. As an aid to investigating these effects in patient samples and to better understand the regulatory mechanisms, we are developing monoclonal antibodies to human thymidylate synthase

#### 4. Preclinical AIDS project

The dihydropteroate synthetase (DHPS) enzyme has been extensively investigated in *T. gondii* organisms. The use of sequential dye affinity chromatographic techniques have been developed to purify the enzyme over 100,000- fold. While the highly purified enzyme was unstable, methods to stabilize the activity have been elucidated. These include the inclusion of excess albumin in the enzyme preparations to prevent adherence to glass and plastic surfaces of the DHPS enzyme and the reducing reagent dithiothreitol. These additions have resulted in stabilization of the enzyme activity for up to 14 days. The purified enzyme has been characterized as having a molecular weight in its native state of 125,000 daltons and an acidic isoelectric point of 6.3. Over twenty sulfonamide and over 40 sulfone analogs have been screened for inhibitory activity against this enzyme. The sulfone compounds were unexpectedly the most potent class of analogs with typical inhibitory constants  $< 1 \mu\text{M}$ . The potency of dapsone, the only sulfone analog available for clinical use, was exceeded by only two new analogs. The sulfonamide derivatives were somewhat less potent than the sulfones and their inhibitory potency varied by over 3 orders of magnitude. The inhibitors with the greatest inhibitory potential were also tested with our intact cell methodology which measures

the incorporation of radiolabeled pABA into the reduced folate pools as an indicator of inhibitor potency. These methods were an outgrowth of the methods developed for the examination of folate pools in cancer cells and have been translated for use in examining folate pools in the various organisms. These studies have corroborated the findings in the cell-free experiments with respect to the interactions of sulfones and sulfonamides with *T. gondii* and *P. carinii* organisms.

In addition to serving as a measure of inhibitory ability of antifolate compounds, we have found this system to be an excellent measure of inhibitory activity of essentially any antimetabolite. As such, we have screened a number of pentamidine analogs supplied by Dr. Peter Tidwell for anti-pneumocystis activity. In addition, we have found the pABA uptake method to be a reliable measure of organism viability. Such a measure is not otherwise available for organisms such as *P. carinii*. This measure has allowed a careful study of potential methods for *in vitro* growth of the organisms.

The tools of molecular biology have been applied to the study of the *P. carinii* organisms as a means of understanding the basic biology of the organism and as a means of obtaining large quantities of recombinant enzyme for drug screening and enzyme characterization. In collaboration with Dr. Jeffrey Edman, we have found been able to isolate and sequence the *P. carinii* ribosomal RNA. This sequence is well studied for a great number of organisms and allows a precise description of taxonomy. Contrary to popular opinion, the *P. carinii* ribosomal RNA is most closely allied with that of the fungi rather than the protozoa. The association with fungi was suspected from our previous studies indicating that the *P. carinii* organism contained a mono-functional dihydrofolate reductase enzyme of low molecular weight (20,000 daltons) rather than the expected high molecular weight bi-functional enzyme (containing thymidylate synthase activity) typical of the protozoa. In addition, these investigations have resulted in the isolation and expression of recombinant *P. carinii* dihydrofolate reductase and thymidylate synthase which are in the process of being characterized and will be available for additional drug screening.

### Clinical

The oral bioavailability studies of trimetrexate glucuronate have been completed in AIDS patients. The fractional absorption of the drug was found to be 0.4 and consistent from patient to patient. The dose escalation study designed to define the optimal dose on the daily schedule was completed in collaboration with Dr. Fred Satler. We found that a dose of 45 mg/m<sup>2</sup> was well tolerated and resulted in 23 of 25 patients having a favorable outcome to the therapy. Doses of 60 mg/m<sup>2</sup> resulted in a highly efficacious regimen (9/9 responses) but was associated with excessive myelotoxicity requiring increased doses of leucovorin and transient discontinuations in therapy.

The blinded, randomized trial comparing trimetrexate with standard therapy for the treatment of *P. carinii* pneumonia is ongoing with a total patient accrual of approximately 200 patients. An interim analysis of the data is scheduled for the spring of '89. The target accrual is set at 370 total evaluable patients.

An oral prophylaxis study based on our preclinical finding that dapsone was a highly potent DHPS inhibitor has been designed and is awaiting final approval by the institutional review board for implementation. This regimen consists of weekly oral pyrimethamine and daily oral dapsone.

In an attempt to enhance the efficacy of trimetrexate, we have initiated a trial of trimetrexate in combination with either oral dapsone or aerosolized pentamidine for the treatment of pneumocystis. At the present time 6 patients have been enrolled onto this study. Finally, since trimetrexate is not available as an oral agent at this time, we have designed a trial for the treatment of *P. carinii* pneumonia using pyritrexim as a substitute for trimetrexate. Pyritrexim is a non-classical antifolate similar to trimetrexate in structure and inhibitory potency with respect to the *P. carinii* DHFR. This compound is was made available from the Burroughs-Wellcome company in an oral formulation whose bioavailability has been well studied.

### 5. Biochemical modulation of Antimetabolites

In addition to Leucovorin, we have identified cis-Platin as an agent capable of positive interaction with 5-FU. This interaction has been characterized in several cell lines and we are presently in the process of defining the mechanisms of this potentially useful interaction. preliminary studies suggest that the locus of the interaction is at the level of DNA repair rather than at the level of protein interactions or metabolic alterations

We have also identified sequence-dependent interactions between 5-FU and ara-azacytidine, a potent new cytidine analog with activity against adenocarcinoma of gastrointestinal origin. Finally, efforts are continuing to identify new agents for the treatment of gastrointestinal malignancies and to understand their mechanisms of action. Cyclopentenyl cytosine is one such agent and we are presently in the process of clarifying its mechanism of action so that it may be applied clinically in a scientifically sound fashion both alone and with other agents.

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Shiroki J, Allegra CJ, Inghirimi G, Chabner BA, Yarboro C, Klippel J. High-dose intravenous methotrexate with leucovorin rescue in rheumatoid arthritis. *J Rheum* 1988; 15:251-5.

Baram J, Chabner BA, Drake JC, Fitzhugh AL, Sholar PW, Allegra CJ. Identification and biochemical properties of 10-formyl dihydrofolate, a novel folate found in methotrexate-treated cells. *J Biol Chem* 1988;263:7105-11.

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## NOTICE OF INTRAMURAL RESEARCH PROJECT

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## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Drug Resistance in Human Tumor Cells

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Craig Fairchild, Ph.D.	Staff Fellow	MB, COP, DCT, NCI
S. Percy Ivy, M.D.	Biotechnician	MB, COP, DCT, NCI
Masayuki Nakagawa	Visiting Fellow	MB, COP, DCT, NCI
Alan Townsend, M.D.	Biotechnician	MB, COP, DCT, NCI
Jeffrey Moscow, M.D.	Clinical Associate	MB, COP, DCT, NCI
Mary Jane Madden, M.D.	Chemist	MB, COP, DCT, NCI
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Medicine Branch

## SECTION

Medical Breast Cancer Section

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6.0

## PROFESSIONAL:

4.0

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This laboratory has been investigating genetic and biochemical changes associated with drug resistance in human tumors. We have characterized an adriamycin resistant human breast cancer cell line which has developed the phenotype of multi-drug resistance. Resistance is associated with decreased drug accumulation (2-3 fold), increased activities of glutathione peroxidase (12 fold), glutathione transferase (45 fold), decreased expression of aryl hydrocarbon hydroxylase (cytochrome P1-450). We have isolated cDNA clones from this resistant cell line which encode the gP 170 membrane glycoprotein, a gene which is often associated with the development of drug resistance. We have also cloned the cDNA for the anionic glutathione transferase GST-pi which is transcriptionally activated in the AdR MCF-7 cells. We have begun a study investigating the expression of the P-glycoprotein gene as well as the expression of several drug metabolizing enzymes including the anionic glutathione transferase GST-pi in the development of clinical drug resistance.

## Multidrug Resistance in Human Breast Cancer Cells

We have been studying the molecular genetic and biochemical changes in multidrug resistant breast cancer cells selected in our laboratory. Resistance in these cells is associated with 2-3 fold decrease in drug accumulation, increased *mdr1* (p-glycoprotein gene expression) increased expression of glutathione peroxidase and glutathione transferase activity, and decreased expression of aryl hydrocarbon hydroxylase (cytochrome P450IA1). These changes are remarkably similar to those which are induced by carcinogens in rat hyperplastic liver nodules (Salt-Farber model) and which are associated with resistance to many structurally unrelated hepatotoxins in that system. These studies suggest that the mechanisms associated with de novo and acquired drug resistance may be the same.

### A. Glutathione S-Transferase

We have recently shown that multidrug resistance in MCF-7 breast cancer cells selected for primary resistance to adriamycin is associated with an increase in glutathione S-transferase activity. This increase is due to the induction of the anionic isozyme of GST (GST  $\pi$ ). We have purified this isozyme and generated polyclonal and monoclonal antibodies against it. Following affinity purification, the polyclonal antibody reacts with a single protein on Western blot analysis. This antibody is being used to screen human tumor specimens for GST- $\pi$  expression using immunohistochemical staining and Western blot analysis. Monoclonal antibodies against GST  $\pi$  purified from AdrR MCF-7 cells have been generated in collaboration with Raphe Kantor at FCRF. These antibodies have been used to screen different epitopes of anionic GST in order to compare the iso-zymes produced in different tissues.

Jeffrey Moscow has isolated GST cDNA clones from a library constructed by Merrill Goldsmith from AdrR MCF-7 RNA. This gene code for a 750 bp mRNA which is overexpressed in AdrR cells. Mary Jane Madden has sequenced this gene and shown that its sequence is remarkably homologous to the rat anionic human GST P gene but differs markedly from the human basic GST genes. This was reported in **Cancer Res**, 1988.

The human GST  $\pi$  cDNA has been used to screen RNA from normal and malignant human tissues. GST  $\pi$  is expressed in 24/26 human colon cancers but in only 2/10 normal colon tissue samples. This gene may thus represent a marker of the de novo resistance of chemotherapy, in this carcinogen-induced tumor. Other studies have shown that GST  $\pi$  expression is increased in 2 patients with recurrent pre B cell ALL relative to the expression in 3 patients with pre B cell ALL at initial presentation. Thus, GST  $\pi$  expression may be a useful marker in acquired resistance to chemotherapy.

We have also found a relation between GST  $\pi$  expression and the absence of estrogen receptors in breast cancer cell lines using Western and Northern blot techniques. Similarly, GST  $\pi$  expression also related inversely to expression of estrogen receptors in primary breast cancer. GST  $\pi$  RNA is moderately high in ER negative (<10 fmoles/lung) breast cancers but is present in low or undetectable levels in ER positive breast cancers. Thus, this gene may be a marker of hormone resistance in breast cancer and may be a useful prognostic marker in this disease. Whether this reflects any difference in chemosensitivity is unclear. This work was reported in **PNAS**, 1988.

Dr. Moscow has developed eukaryotic expression vectors which express GST  $\pi$  in transfected cells. MCF-7 cells transfected with this vector, over expressing GST  $\pi$  are resistant to the carcinogens benzo(a)pyrene and benzo(a)pyrene antidiol epoxide. These cells display little change in sensitivity to adriamycin, cisplatin, and phenylalanine mustard. Thus, the role of this gene is antineoplastic drug resistance is not clear. This work is in press in **Mol. Pharm.**, 1989.

## B. GST $\pi$ Gene Regulation

Since  $\pi$  class GST is overproduced in two models of drug resistance, the multidrug resistant MCF-7 breast cancer cell line and the Salt-Farber resistant hyperplastic liver nodule system, our laboratory has focused attention on what regulates the expression of this gene. In order to do this, Dr. Charles Morrow in our laboratory has cloned the human genomic GST  $\pi$  gene and has sequenced the entire gene as well as 2500 base pairs of 5' flanking sequence. In order to study the elements involved in transcriptional regulation of this gene, Dr. Morrow has fused the 5' flanking sequences of the human genomic GST  $\pi$  gene with the reporter gene bacterial chlorophenylcol acetyl transferase (CAT). Dr. Morrow made a series of 5' end deletions and internal deletions mutants of the GST  $\pi$ -CAT fusion constructs. These constructs were transfected into a series of cell lines and Dr. Morrow has been able to identify at least two different elements which are involved in the transcriptional regulation of this gene. Furthermore, Dr. Morrow has found that the human GST  $\pi$  gene like the rat GST-P gene, has a consensus sequence which corresponds to the binding site for AP-1 transcription factors, which include the c-jun oncogene. These sequences are normally upstream from genes which are regulated by phorbol esters. In order to study whether this sequence is indeed involved in the regulation of the human

GST  $\pi$  gene, Dr. Morrow has co-transfected the human GST  $\pi$ -CAT fusion constructs with expression vectors containing the jun oncogene with or without vectors expressing the fos oncogene. (In other systems, the fos protein has been shown to interact with the jun oncogene to augment expression of genes from these AP-1 consensus site). Preliminary data suggest that the AP-1 site in the human GST  $\pi$  gene is not recognized by the c-jun oncogene. This may be related to other protein binding sites immediately downstream from the site in the human GST  $\pi$  gene. The work on cloning and sequencing of the human GST  $\pi$  gene has been published in **Gene**, 1989 and a second manuscript on the regulation of expression of the GST  $\pi$  gene is in preparation.

## C. P- Glycoprotein - mdr gene

Craig Fairchild, and Percy Ivy, and Merrill Goldsmith have isolated P-glycoprotein cDNA sequences from AdrR MCF-7 cells. This gene is overexpressed in most multidrug resistant cell lines. Its expression in human tumors is under investigation.

As alluded to previously, studies from our lab have shown that many biochemical changes in MDR MCF-7 cells are similar to those that are associated with xenobiotic resistance in carcinogen-induced rat hyperplastic liver nodules. Further studies have now shown that xenobiotic resistance in rat HNS and hepatomas is associated with overexpression of P-glycoprotein gene. Moreover, acute treatment with acetlyamino-fluorene and partial hepatectomy results in over an 80 fold induction of P-glycoprotein expression. This acute induction of P-glycoprotein represents an useful model to examine the regulation of P-glycoprotein gene expression. This work was published in **PNAS**.

Dr. Fairchild has sequenced the *mdr-1* overexpressed in adriamycin-resistant MCF-7 cells and found one nucleotide change resulting in an amino acid substitution in a trans-membrane domain of the *mdr-1* gene in adriamycin-resistant MCF-7 cells. This is of interest since previous studies by Roninson and coworkers have shown that a single amino acid change in the *mdr* gene in another region apparently encodes a P-glycoprotein with a different phenotype and resulting in an apparently altered binding affinity for colchicine. Whether the amino acid difference in the adriamycin-resistant MCF-7 cells encodes for a protein with an altered phenotype is currently being investigated. Dr. Fairchild has created an expression vector using *mdr-1* gene sequences isolated from AdrR MCF-7 cells. These were fused to a variety of different promoters and each of the expression vectors have been transfected into wild type MCF-7 cells. The *mdr-1* expression vectors do result in a multidrug resistant phenotype when transfected into drug sensitive cells. The phenotype is similar to that present in the parent cell line (AdrR MCF-7). Since we have found overexpression of both the P-glycoprotein and the GST  $\pi$  genes in multidrug resistant MCF-7 cells, we have also examined whether these two genes can function together. Cells transfected with *mdr-1* expression vector only were then subsequently transfected with the GST  $\pi$  expression vector created in our laboratory. Clones were selected by co-transfected with PSV-Neo and surviving colonies were screened for glutathione transferase activity. Subclones expressing high levels of GST  $\pi$  were then grown and studied for drug resistance. Overall, we could find no difference between the pattern and level of multidrug resistance in *mdr-1* transfected cells and not expressing GST  $\pi$  compared to those subclones which express high levels of GST  $\pi$ . Therefore, we conclude that GST  $\pi$  does not apparently interact with the mammalian P-glycoprotein. The basis for this work had been derived from sequence homology between the mammalian P-glycoprotein and bacterial membrane transport genes. In the bacterial system, the membrane transport proteins are in general a multicomponent system including not only a membrane protein and an energy-generating protein, but also at times a periplasmic binding protein which binds ligand and is subsequently transported as a complex through the membrane protein. Since glutathione transferases are known to be high affinity binding proteins, the possibility that the GST  $\pi$  protein was involved for transport through the P-glycoprotein had to be considered. As listed above, our current studies do not support this hypothesis. This work has been submitted to JBC for publication.

#### D. Cytochrome P450IA1 Regulation.

Dr. Percy Ivy in our laboratory has been involved in studying the down regulation of cytochrome P450IA1 gene, which encodes the enzyme activity aroclorhydrocarbon hydroxylase. This gene is down regulated in our drug resistant multidrug resistant MCF-7 cells as well as in the rat hyperplastic liver nodule system. We have shown that the gene is inducible by polycyclic hydrocarbons such as tetra-chlorodioxane (TCDD), but not inducible in the AdrR MCF-7 cells. Since we can find little, if any difference in putative Ah receptors (TCDD-binding proteins) in the wild type and AdrR MCF-7 cells, the defect is not apparently at the level of quantitative receptors. This also not a promoter defect, since transfection of a mouse P450IA1 promoter-CAT construct is inducible on the wild type MCF-7 cells but not in the AdrR MCF-7 cells. Dr. Phil Vickers in our laboratory was able to show that there is a correlation between estrogen-receptor content and inducibility of P450IA1 in human breast cancer cells. This gene is more inducible in estrogen

receptor-positive tumor cell lines than it is in estrogen receptor-negative tumor cell lines. This also did not appear to correlate with the quantitative estimate of TCDD binding proteins as there are levels in estrogen receptor negative breast cancer cell was in general as high as the level in estrogen receptor positive breast cancer cell lines. Studies were published in *JBC*, 1988 and *Mol. Endo.*, 1989. We are continuing our studies on the regulation of cytochrome P450IA1 in multidrug resistant cell lines in order to identify the factors involved in the regulation of the expression of this gene. Since this gene is an important metabolic pathway for the activation of carcinogens, and since the P-glycoprotein gene has been recently shown by S. Thorigesen and coworkers to be inducible also by polycyclic hydrocarbons must understanding the regulation of the P450IA1 gene may have important implications for carcinogenesis as well as drug resistance.

#### E. Alteration in Hormonal Sensitivity in MDR Breast Cancer Cells

MDR breast cancer cells have become cross resistant to antiestrogens. Phil Vickers in our lab in collaboration with Robert Dickson in the Medicine Branch has examined the hormonal sensitivity of AdrR MCF-7 cells. In contrast to the parental MCF-7 cells, estrogen (E2) does not increase, nor does the antiestrogen tamoxifen decrease the growth of AdrR MCF-7 cells. Moreover, while E2 induces secretion of specific polypeptides from parental MCF-7 cells and induces progesterone receptors in that cell line, E2 produce neither effect in AdrR MCF-7 cells. This lack of hormone sensitivity of MCF-7 cells selected for adriamycin resistance is associated with a loss of estrogen receptors as measured by hormone binding and antibody precipitation analysis. In contrast, membrane EGF receptors are markedly increased in the AdrR MCF-7 cells and is associated with enhanced tumorigenicity in nude mice. While WT MCF-7 cells will form tumors in nude mice only in the presence of exogenic estrogen administration, the multidrug resistant subline readily forms tumors in the absence or presence of estrogen. Thus, this drug resistant cell line is estrogen independent in vitro or in vivo. This work was published in *Mol. Endo.*, 1988.

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## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06519-06 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Non-Invasive Studies of Metabolism Using Nuclear Magnetic Resonance Methods

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Patrick Faustino, M.Sc.	Chemist	MB, COP, DCT, NCI
Peter van Zijl, Ph.D.	Visiting Associate	MB, COP, DCT, NCI
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4.0

## PROFESSIONAL:

4.0

## OTHER

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

We wish to understand differences in the regulation of metabolism between normal and cancer cells and between cancer cells that are responsive to chemotherapy and those that exhibit multiple drug resistance (MDR). Nuclear magnetic resonance (NMR) spectroscopy is used to monitor metabolic processes non-invasively in vitro, using a perfusion technique that we have developed in which cells are embedded in gel threads and in vivo. With <sup>31</sup>P NMR, we have observed significant differences in the levels of major phosphate metabolites in wild type (WT) NCF-7 breast cancer cells and an adriamycin resistant cell line (AdrR) that exhibits MDR. Phospholipid metabolism in these cells has been monitored from the levels of phosphomonoester (PME) and phosphodiester (PDE) peaks in the <sup>31</sup>P NMR spectra. We have found that the higher the PME peak, the more rapidly the cells proliferate. These results may provide spectroscopic markers for clinical distinction of growths. One of the major differences between normal and cancer cells is the control of energy metabolism. To follow glycolysis in WT and AdrR cells, we use <sup>13</sup>C-labeled glucose and monitor the rate of glucose uptake and the concomitant rate of lactate production with <sup>13</sup>C NMR. The results showed that the AdrR cells are more dependent on glycolysis than the WT. The effects of 2-deoxyglucose, an inhibitor of glycolysis on cell growth and metabolism has been studied. The results indicate a selective cytotoxicity for the AdrR cells, indicating that energy anti-metabolites may be useful agents against MDR. To extend the observations to non-invasive metabolic measurements in vivo, we have designed and constructed a versatile NMR probe for our spectrometer. We observed spectra from subcutaneous human breast tumors in nude mice. Using the 4.7 Tesla in vivo spectrometer in the NIH NMR Center, we focused on developing localized proton NMR spectroscopy. This has also been achieved in a collaborative effort for the Signa 1.5 T whole body system, and we are obtaining excellent spectra from 1 ml volumes from inside tumors of patients.



## MAJOR FINDINGS

### Effects of 2-Deoxyglucose on Drug-Sensitive and Drug-Resistant Breast Cancer Cells: Toxicity and Magnetic Resonance Spectroscopy Studies of Metabolism

The glycolytic inhibitor 2-deoxyglucose (2-DG) was tested as a potential chemotherapeutic agent for drug resistant human breast cancer cells. Previously it was found that adriamycin-resistant human MCF-7 breast cancer cells (ADR) exhibit an enhanced rate of glycolysis compared to their parent wild-type (WT) cell line (Lyon, et.al., *Cancer Res.* **48**: 870-877, 1988). We have now found a specific toxic effect of 2-DG on the ADR cells, which is more than 15-fold greater than for WT cells.

Using  $^{31}\text{P}$  magnetic resonance spectroscopy (MRS) of perfused MCF-7 cells, we continuously monitored the accumulation of 2-deoxyglucose-6-phosphate (2-DG-6P), together with concomitant changes in other phosphate-containing metabolites. Kinetic measurements demonstrated that ADR cells accumulated 2-DG-6P faster and to a greater extent than WT cells, while their depletion of high energy compounds (ATP, PCr) was more pronounced and became irreversible earlier.

The phosphorylation of 2-DG could be followed more effectively by the use of  $^{13}\text{C}$  at the C6 position, since the signals of 2-DG and 2-DG-6P are clearly resolved and unlike  $^{31}\text{P}$  MRS, there are no other interfering signals. Employing this technique with ADR and WT cells, the rates of phosphorylation of 2-DG and 2-DG-6P were found to be  $11.2 \times 10^{-4}$  and  $6.5 \times 10^{-4}$  mM/min/mg protein, respectively. The results of these studies indicate that differences in the energy of biochemistry of energy metabolism of resistant cells may make them targets for energy anti-metabolites. We have started a series of studies, using NMR methods in which the tumor cells are treated with antimetabolic agents (rhodamine 123, gossy-pol) and 2-DG, in order to find a useful therapeutic combination with minimal toxicity in normal cells.

### Epidermal Growth Factor (EGF) Effects on MDA-468 Human Breast Cancer Cells (In collaboration with Dr. Zugmaier and Bruce Ennis, Lombardi Cancer Center, Georgetown University).

EGF was found to be toxic to specific tumor cells grown in cultures.  $^{31}\text{P}$  NMR studies of perfused MDA-468 cells in agarose threads demonstrated no ATP decrease, and it was found that the cells remained viable after prolonged (20 hours) perfusion with high EGF concentrations. However, by using these NMR techniques, it was found that EGF enhanced 2-deoxyglucose (2-DG) uptake and phosphorylation in MDA-468 cells. We are now performing survival assays of these cells incubated in various glucose, 2-DG, and EGF concentrations, as well as studies of the activity of the glucose enzymes with and without EGF. These studies would hopefully provide data on the mechanism of EGF activity.

### Studies of the Biochemical Changes in Transformed Lymphocytes and Tumor Infiltrating Lymphocytes (TIL) (In collaboration with the Surgery Branch, NCI).

In order to investigate the changes that occur in lymphocytes during their activation, and to define the specific TIL cells,  $^{31}\text{P}$  NMR studies of normal peripheral lympho-

cytes, LAK cells, and TILS perfused in agarose threads are performed. Preliminary results have demonstrated substantial differences between the various lymphocyte types. For example, TILS have which levels of glycerophosphocoline and glycerophosphoethanolamine which were not found in LAK cells. These compounds have a role in membranous phospholipid metabolism. Lymphocytes are characterized by a dominant phosphoethanolamine signal and perfusion with high choline concentrations result in the appearance of phosphocholine peak and a currently unidentified new peak. We plan to perform P31 and H1 NMR studies of various types of intact lymphocytes, extracts of lymphocytes and use agents that modify metabolic pathways in order to define the biochemical changes in activated lymphocytes.

#### The Effects of Tumor Necrosis Factor (TNF) on MCF-7 Cells (In collaboration with Dr. John Fruehauf, MB)

It was reported that TNF injections to tumor bearing mice caused reduction in ATP levels and tumor size. However, the mechanism of TNF effects were not clear. In order to discover whether there is a direct metabolic effect on tumor cells and ATP depletion, P31 NMR studies with additional TNF were performed. Preliminary results exhibited no change in the P31 spectra, indicating that TNF may act through neoendothelial damage leading to thrombosis and secondary ischemia. We plan to continue these experiments using various TNF concentrations in the incubating medium, and in the perfusate.

#### High Field Localized Proton Spectroscopy in Small Volumes: Greatly Improved Localization and Shimming Using Shielded Strong Gradients

Recently, very high resolution proton NMR spectra have been obtained *in vivo* using the stimulated echo volume localization technique (stimulated echo acquisition mode, STEAM). However, these results have been limited mainly to low fields (1.5 T-2 T) and to volumes of at least a few cubic centimeters located within large tissue masses with minimal susceptibility effects, e.g., the human brain. In most animal models, on the other hand, regions of interest are generally restricted to volumes smaller than 1-2 cm<sup>3</sup>. As a consequence, neighboring tissues of different magnetic susceptibilities complicate accurate localization and shimming of the volume of interest (VOI). These problems become increasingly important at higher field and form the basis for widespread skepticism regarding the feasibility of high field resolution NMR *in vivo*. We have demonstrated that the above problems can be reduced significantly when shielded strong gradients are available for  $B_0$  localization procedures. Attention will be focused on the STEAM sequence. The high performance gradients serve many purposes in producing optimum results with this sequence, that is, in obtaining a pure stimulated echo of the VOI only.

The availability of shielded gradients facilitates accurate single-voxel localized spectroscopy. Exact localization (90-99%) of volumes as small as 2 x 2 x 2 mm is demonstrated using a modified stimulated echo sequence. Strong gradient pulses (up to mT/m) are employed to clear the stimulated echo of unwanted magnetization, enabling superior (single-scan) shimming of the selected volume. *In vivo* and *in vitro* proton NMR results at 4.7 T have been obtained (see below).

In order to improve this technique, certain technical innovations have been carried out by Dr. Peter VanZijl in collaboration with Dr. Chrit Moonen of the NIH NMR Cent Improved Water Signal Suppression Technique for *In Vivo* NMR Center.

Water suppression techniques are generally divided into two categories: 1) those that selectively avoid exciting the solvent spins before acquisition of the free induction decay (e.g. rapid scan correlation spectroscopy and selective excitation and 2) those that destroy longitudinal solvent magnetization before generation of the FID (e.g. inversion recovery and saturation. To these should be added techniques based on 3) transverse relaxation or on 4) scalar coupling properties (multiple quantum schemes). Most of the techniques in the first two categories are based on a difference in chemical shift between solute and solvent, having the direct disadvantage that they depend on water linewidth and that resonances close to or at the water frequency cannot be studied. Sequences using properties like relaxation ( $T_1$  and  $T_2$ ) or scalar coupling do not suffer from these drawbacks. Disadvantage of pure relaxation suppression is that differences between solvent and solute may not be large enough for certain groups of spins, leading to relaxation-dependent signal ratios. Problems with multiple quantum techniques are loss of signals with single quantum properties, the need for phase cycling (except when gradients are available), signal loss due to evolution time and signal intensities that are a function of chemical shift (differences/sums).

According to the best knowledge, all presently available suppression schemes are based on magnetic spin properties. A technique exploiting other physical properties of the nuclei under study will have direct advantage of the many parameters in the rf excitation scheme and of not suffering from  $B_1$ -homogeneity. Since such a method will be independent of chemical shift, water linewidth will not play a role in the success of the actual suppression scheme and spins resonating at the water frequency can be studied. We have developed a suppression technique that falls in this category. It relies only on differences in diffusional properties between water (or any solvent) and the solutes.

Experiments were performed using the 4.7 T wide bore animal imager (GE CSI) equipped with acustar shielded gradients. Spectra with perfect suppression of water were obtained with a spin echo sequence.

#### Localized Proton Spectroscopy of Tumor Cells

Proton NMR spectroscopy of tumors has been performed on extracts, excised tissue and suspensions. However, extraction and excising procedures may alter the biophysical state of the cells. In order to follow prolonged metabolic processes under physiological conditions, continuous perfusion experiments are necessary, a procedure recently applied for  $^{31}\text{P}$  NMR studies. We have now extended this work to  $^1\text{H}$  NMR, using the stimulated echo (STEAM) for localized spectroscopy. This has the advantage that the same technique can be used *in vivo*, with greater potential significance. Also, a volume of interest (VOI) can be localized in the cell suspension only, avoiding susceptibility broadening related to the perfusion system and the container glass. A further advantage is that STEAM can be combined with effective water suppression. As a first example, we report spectra of human MCF-7 wild type breast carcinoma cells (WT) as well as of the adriamycin resistant (AdR) cell line derived from them.

Cells were grown in minimal Eagle's medium to 80-95% confluency, harvested by trypsinization and cast in a 50/50 cell agarose suspension (density:  $1.5 \times 10^8$  cells/ml) in a perfusion vial. Continuous perfusion with growth medium occurred at a rate of 0.7 ml/min. A VOI of 0.5 ml was localized and shimming was performed on the water signal, after which it was suppressed. A solenoid (1.2 cm) around the bottle was used to transmit and receive. Field strength was 4.7 T. Spectra of medium only under the same conditions did not show any signal.

The spectra for WT and AdR type cells showed striking differences. However, without a standard, quantitative comparisons are difficult to make. e noted that a ration of some signals are about the same in both spectra. Assuming no change in their intensities and using them as a reference, some conclusions can be drawn. First, the intensity of a signal is strongly reduced for AdR suggesting a different membrane structure or reduced lactate production. Differences in this region have been reported by others, who related them to metastatic potential. Secondly, signal intensity in the glutamate/glutamine region is altered, which may point to different energy metabolism. Thirdly, there are changed intensities in the 3.0-3.5 ppm region, where choline and ethanolamine resonate, which may be indicative of membrane function also. Other resonances which appear in this region could be taurine, glutathione, inositol, and glycine. Fourthly, the region between 5.0 and 8.5 ppm, in which exchangeable amine/admide protons resonate is drastically different. At present, we are performing echo-time dependent studies as well as experiments with different perfusates to obtain more insight on the assignments and metabolism.

Localization makes proton NMR studies of perfused cells feasible. AdR and WT cells show characteristic spectra, which is of potential interest for diagnosis of tumors *in vivo* using the same localization technique. Other possible applications are the development of new therapeutic modalities, in particular against drug resistant tumors, and the study of malignancy.

#### Localized Proton Spectroscopy of Superficial Tumors in Humans. Spectral Changes upon Radiation Treatment of Squamous Cell Carcinoma

Recently, substantial interest has arisen in the potential of localized proton spectroscopy for the study of human metabolism. Most studies have been performed STEAM or ISIS on large volumes ( $> 15$  ml) in homogeneous tissue like brain or muscle. One study using ISIS was done for a 1 ml voxel in brain. To be able to characterize tissue, one has to study homogeneous regions. It is therefore of utmost importance to go to small volumes, especially for diagnosis of tumors, which are generally quite heterogeneous. Also, upon treatment, these tumors may shrink, making it not very useful to study the same large volume over a period of time. In this respect, we wanted to evaluate the potential of STEAM for the study of homogeneous voxels in tumors.

A first test study was performed on a patient with a squamous cell carcinoma nodal metastasis in the neck. Before treatment, tumor volume was about 200-250 ml, which fell to 25-40 ml after treatment with continuous infusion of iododeoxy-uridine (putative radio sensitizer) and radiation (39 Gy). In both situations, volumes of 1-3 ml could be localized in homogeneous tissue. Homogeneity was judged from gradient recalled echo images. Fig. 1 shows a spectrum at TE = 25 ms, taken 1 week after the start of treatment (918 Gy). Spectral appearance resembles that of cell suspensions and extracts of other tumor types and recent data on brain tumors and perfused cells. The two peaks low field of Cho disappear at longer echo times. Spectral appearance did not change

quantitatively before treatment and the first three weeks during treatment, although the tumor was already shrinking. After 4 weeks, a dramatic change occurred in the disappearance of the metabolites. We tentatively contribute this to cell destruction and flushing out of intracellular metabolites.

Proton NMR spectra of a 1 ml voxel localized in a superficial tumor are reported for the first time. A first study of treatment response diagnosed by proton NMR is presented for a squamous cell carcinoma upon radiation treatment. This single case showed no spectral evidence of changed metabolism prior to reduction in tumor size. However, more cases will have to be studied and different tumor types/treatments may give varying results. Also, the ability to detect loss of intracellular metabolites, while a mass still remains, may also be useful to determine fiber/necrotic masses from residual tumor.

### Single-shot Imaging *In Vivo* at 4.7 Tesla of Localized Selected Volumes

A frequently encountered problem with echo-planar single-shot imaging is the requirement of extremely good static field homogeneity in order to obtain acceptable artifact-free images. With a typical acquisition time of 10 microseconds per point for a 64 x 64 image, this is equivalent to a linewidth of 25 Hz or better, assuming the linewidth is not dominated by T2. Thus, at 4.7 Tesla, a homogeneity of better than about 12 parts per billion is required over a distance of perhaps 50 mm. For *in vivo* imaging, where tissue may be quite heterogeneous and the susceptibility may vary by parts per million, it is not easy to shim to this accuracy, and hitherto, it has been possible to obtain single-shot images only of simple objects such as a hen's egg.

For some time it has been known that over localized volumes of interest within an object it is possible to adjust the field to obtain remarkably high homogeneity. This would suggest that a combination of localization and single-shot imaging could facilitate the acquisition of excellent single-shot images. A two-pulse "zoom" technique was developed which has been successfully used to image blood vessels.

However, there are considerable advantages, including ease of shimming and freedom from motion artifact, to be gained from single-shot volume localization. At present, the most straightforward such techniques uses the stimulated echo. We have implemented this method (STEAM-EPI), and obtained good single-shot images of localized volumes of the cat's brain with an in-plane resolution of 0.5 mm and a slice thickness of 2 mm. The instrument used was the GE CSI system, equipped with Acustar shielded gradients, at a static field of 4.7 Tesla.

The image-forming gradients were at first switched off, and the shim was performed using only first-order shims over the selected box-shaped volume, which can be of any proportions anywhere in the object. For convenience, a thin rectangular slice was taken, and it was easy to shim to a linewidth of a few Hertz (limited mainly by tissue T2). Standard echo-planar imaging gradients were then switched on, enough data (4096 complex points) for a 64 x 64 image were acquired, and the image was reconstructed in a few seconds using an on-line array processor. When signal:noise ratio was judged inadequate, it was simple to improve it sufficiently by averaging over a few acquisitions. When the volume was not located about the magnet isocenter, it was necessary to offset the reference frequency in order to avoid the image artifact.

Unavoidably, the use of the stimulated echo incurs the loss of up to half the signal. At 4.7 Tesla, this is not a severe problem; nonetheless, other localization techniques are being assessed which avoid this signal loss.

### Instrumental Improvements in NIH NMR Center

#### 4.7 T *In Vivo* Spectrometer

A. The following projects are in operation:

15 cm diameter Birdcage paddle wheel design coil.

This coil has a unique low eddy current RF shield. The coil lines and matches over a large variation of load conditions. The commercial version sent by the manufacturer did not work under all load conditions.

15 cm  $H_1/C_{13}$  Na Spectroscopy and imaging coil

6 cm $H_1$ surface coil	}	These are of a new design
5 cm     "      "	}	which show little electric
3 cm     "      "	}	field loss

8 cm diameter Birdcage imaging and hyperthermia coil for *in vivo* treatment/heating of small animal tissue.

A variety of small volume high resolution coils have been made and tested. These have been both single and multinuclear design. In addition, several rat tumor surface coils have been constructed and tested. Low pass filters for cardiac and respiratory gating have been built and used. The 18 gauss/gradients have been replaced with a 25 cm 3 gauss/cm pet (temporary).

B. Work in progress.

4.7 T design and construction of stereotaxic animal position holders

Quadrature detection system for a square-root 2 increase in signal to noise

Low noise preamplifiers have been acquired and should be installed shortly. We are waiting for hardware delivery from GE. Temperature stabilized cell probe has been designed and is being fabricated by BIB. Preliminary design and construction of a Taseen landmarking and docking system for position reproducibility.

Several transmit and receive channels for use with volume and high sensitivity surface coils. These will be switched with PIN diodes.

#### 7 T Spectrometer

The rat probe NMR probe body is complete and waiting animal protocol approval before the RF electronics are installed.

In collaboration with Robbe Lyon, a rat brain probe with multinuclear capability Na, K, P,  $H_1$  has been designed and used.

2 T Spectrometer

A host of small volume coils and surface coils have been built.

David Place's small animal imager has proved reliable and quite useful in the contrast agent diffusion perfusion studies.

A passive transmit/receive system has been designed in collaboration K. Hedges. This employs passive decoupling circuits that prevent coupling of the receiver to the transmitter.

Computer modeling of Eddy current compensation circuits with Alan Olson and R. Tunnen. These have reduced the acoustic resonance problems in the gradient coils by an order of magnitude.

A variety of birdcage designs have been built and evaluated. The spherical chamber version is showing promising results in sensitivity and RF field uniformity. This should have immediate applications for clinical use. Computer modeling of these periodic resonant structures is under way using finite element analysis.

Signa Clinical System

The following coils have been evaluated:

Multiple tuned  $P_{31}/H_1$  spectroscopy and imaging coils. 2.5 cm and 6 cm.

$P_{31}$  saddle coil for *in vivo* spectroscopy. This works within the Signa head coil.

A highly uniform  $B_1$  producing surface coil design has been developed and has been used for volume localization experiments.

Collaborative work on limited RF penetration modalities for the evaluation of skin surfaces (melanomas, wounds, scar tissue formation).

Sodium/lithium imaging systems for drug studies.

A variety of coil designs to prevent unwanted RF deposition during transmission have been developed and tested.

Work in progress:

Quadrature/Orthogonal surface coils antenna arrays, finalization of double tuned Birdcage design.

PUBLICATIONS:

Daly PF, Lyon RC, Straka EJ, Cohen JS.  $^{31}\text{P}$  NMR spectroscopy of human cancer cells proliferating in a basement membrane gel. *FASEB J* 1988;2:2596.

Cohen, J.S.: Phospholipid and energy metabolism of cancer cells monitored by  $^{31}\text{P}$  magnetic resonance spectroscopy: possible clinical significance. *Mayo Clin Proc* 1988;63:1199.

Daly P, Cohen JS. Magnetic resonance spectroscopy of tumors and potential in vivo clinical applications: a review. *Cancer Res* 1988;49:770.

Cohen JS, Lyon RC, Daly PF. Monitoring intracellular metabolism by NMR. *Methods In Enzymology*, in press.

Daly PF, Zugmaier G, Sandler D, Carpen M, Myers CE, Cohen JS. Regulation of the cytidine pathways in human cancer cells and effects of ara-c: a non-invasive  $^{31}\text{P}$  NMR Study. *Cancer Res*, in press.

Van Zijl P, Moonen CWT, Alger JR, Cohen, JS, Chesnick SA. High field localized proton spectroscopy in small volumes: improved localization and shimming using shielded strong gradients. *Mag Res Med* 1989;10:256.

Navon G, Lyon RC, Kaplan O, Cohen, JS. Monitoring the transport of 2-deoxy-D-glucose in tumor cells in vivo and in vitro by  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy. *FEBS Lett* 1989;247:86.

Niemczura WP, Helms GL, Chesnick, SA, Moore RE, Bornemann V. Carbon-detected correlation of carbon-13-nitrogen -15 chemical shifts. *J Magn Reson* 1989; 81:635-40.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06520-06 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Contrast Agents for Magnetic Resonance Imaging of Tumors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jack S. Cohen, Ph.D.	Principal Investigator	MB, COP, DCT, NCI
Patrick Faustino, M.Sc.	Chemist	MB, COP, DCT, NCI
David Place, Ph.D.	Guest Researcher	MB, COP, DCT, NCI
Peter van Zijl, M.D.	Visiting Associate	MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

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## LAB/BRANCH

Medicine Branch

## SECTION

Biophysical Pharmacology Section

## INSTITUTE AND LOCATION

NIH, National Cancer Institute, Bethesda, MD 20892

## TOTAL MAN-YEARS

1.5

## PROFESSIONAL

1.5

## OTHER

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Magnetic resonance imaging (MRI) is an important tool in diagnostic radiology and allows discrimination between a growth and surrounding soft tissue. The contrast depends on the relaxation times (T-1 and T-2) of the bulk water in the respective tissues. A paramagnetic substance is one that contains an unpaired electron and alters the relaxation rate of water. Our objective is to identify a class of paramagnetic contrast agents which selectively are retained by tumors in order to facilitate and clarify MRI tumor contrast.

We have chosen the water-soluble metalloporphyrins (WSMPs) for several reasons: porphyrins are known to be selectively retained by tumors, they form complexes with metal ions, and the synthetic water soluble porphyrins are much more tractable than the hydrophobic natural heme products. We have evaluated several of these WSMPs by several criteria: solubility, relaxivity, stability in human plasma and in vivo, distribution in vivo, toxicity, and MRI contrast properties. As a result of these comparisons, we have identified Mn (III) TPPS as a potential tumor contrast agent in MRI. We are studying the mechanism of tumor retention and stability of porphyrins and WSMPs and quantitative dose-contrast relationships in MRI of nude mice and rat tumors for a series of WSMPs.

## Major Findings

### Manganese-Porphyrin MRI Contrast Agents for Tumors

The long-term goal of this research is to identify and study paramagnetic compounds which concentrate in tumors, thereby enhancing Magnetic Resonance Imaging detection of tumors. Although porphyrins have been used for photodynamic therapy for several decades, we reported that Mn(III)tetra(4-sulfonatophenyl) porphyrin, MnTPPS<sub>4</sub>, a water-soluble paramagnetic metalloporphyrin, is a contrast agent which can effectively enhance tumor detection by MRI (Patronas, N.J., *et al*, *Cancer Treat. Rep.*, **70**, 391, 1986). Its low toxicity, selective accumulation in tumors, and MRI contrast enhancement in tumor-bearing nude mice have also been documented (Lyon, R.C., *et al*, *Mag. Res. Med.*, **4**, 24, 1987). We have continued our studies of this agent in order to further understand the mode of action of MnTPPS<sub>4</sub>. Results from MRI are correlated with experiments performed by Mr. Patrick Faustino on intact tumors and tumor cell cultures. Current experiments center on the determination of dose-contrast relationships over a wide range of dosages in order to determine the lowest dose as well as the best administration method for obtaining visible MRI tumor contrast enhancement. Future work will include studies of other water-soluble manganese porphyrin complexes.

### Instrumentation Advances and MRI Results

Instrumentation advances within the past year at the NIH *in vivo* NMR Center allow much higher image resolution than in earlier research. Spin Echo (SE) and Inversion Recovery (IR) images have been obtained on a 2T GE CSI equipped with shielded gradients. A custom-designed birdcage coil, which accommodates one mouse, is being utilized. The combination of optimized probe design, higher field strength, and state-of-the-art gradients affords high S/N, small pixel size, and effective data acquisition.

Athymic nude mice, bearing subcutaneous MCF-7 human breast carcinoma xenografts were injected intraperitoneal (IP) doses ranging from 0.025 to 0.5 mmol/Kg. Because of the moderate solubility of MnTPPS<sub>4</sub>, the injected volume of agent for high doses exceeded the allowable volume for single-bolus IV injections. IP administration has been chosen to eliminate this difficulty. In addition, we were interested in whether a more gradual vascular uptake and distribution of the agent, such as IP injection provides, would lead to improved contrast enhancement. The anesthetized mice were imaged before and after MnTPPS<sub>4</sub> administration. Images of 2 mm thickness were obtained which include the tumor and a full coronal cross-section of the mouse for image contrast comparison.

In order to determine contrast enhancement, SE images with a range of T<sub>1</sub> and T<sub>2</sub> weighting have been obtained. Substantial systemic relaxation enhancement is observed on T<sub>1</sub>-weighted images shortly after injection, but good tumor enhancement is not apparent until one day after injection, when the background level from the normal tissues has decreased. Individual doses of 0.025 and 0.5 mmol/Kg do not produce visible image enhancement. Single doses of 0.1 mmol/Kg or greater appear to be necessary to produce a detectable effect. At a level of 0.5 mmol/Kg, marked en-

hancement is produced, the effects of which can be readily observed over the course of several days before imaging do not appear to be cumulative in nature. Although the contrast effects of lower doses are readily apparent in the series of SE images, a  $T_1$  difference analysis at short TE values may prove useful for these clinically relevant lower doses. Currently under development with Dr. Peter van Zijl are spatial localization techniques which allow us to determine  $T_1$  and  $T_2$  on volumes as small as 3 mm.

### Biodistribution

The use of a normal human breast cell line as a control to monitor the uptake, retention, and stability of the metalloporphyrin MRI contrast agent has provided insight into the differences in the biodistribution of the drug in cellular organelles of both normal breast cells and MCF-7 human breast cancer cells. In normal breast cells, the drug seems to be primarily membrane-bound indicating that much of the drug is not distributed intracellularly. In cancer cells, the drug seems to accumulate in the mitochondria. Such differences are important and provide a basis for exploiting the possibility of an energy transport or active transport mechanism via proteins or enhanced metabolic rate of cancer cells. Experiments are also being undertaken with an *in vivo* MCF-7 tumor model subcutaneously implanted in mice and injected with potential clinical doses of the drug to determine biodistribution and toxicity.

### Active Transport Experiments

The biodistribution differences between normal breast cells and breast cancer cells may indicate an active transport mechanism. Experiments have been done initially at 4 and 37 degrees in the breast cancer cells to show that some uptake may be a result of active transport as a direct result of temperature. Experiments are underway to exploit this possibility by using metabolic inhibitors of glycolysis and oxidative phosphorylation. Experiments are also being planned to block hem-like receptors on the cell surface.

### Toxicity Experiments

The stability of the metalloporphyrins in biological systems has yet to be quantitatively determined in either *in vivo* or *in vitro* models. The ability to do such experiments has come to fruition in our laboratory with the development of an HPLC assay that effectively separates metalloporphyrins from its more toxic demetallized porphyrin. The HPLC is utilized to separate the two components and fluorometer that has subnanaogram detection of porphyrin is connected to the HPLC to determine porphyrin generation or demetallization of the metalloporphyrin.

### PUBLICATIONS

Van Zijl P, Place D, Cohen JS, Faustino PJ, Lyon RC, Patronas NJ. Metalloporphyrins magnetic resonance contrast agents: feasibility of tumor-specific MRI. *Acta Radiologica*, in press.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06521-06 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Conformations and Interactions of Nucleic Acids, Proteins, and Drugs in Solution

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Jack S. Cohen, Ph.D.

Research Chemist

MB, COP, DCT, NCI

Jerzy Jaroszewski, Ph.D.

Visiting Associate

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## COOPERATING UNITS (if any)

C. Syi and J. Maizel, ASCL, FCRF, NCI

## LAB/BRANCH

Medicine Branch

## SECTION

Biophysical Pharmacology Section

## INSTITUTE AND LOCATION

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## TOTAL MAN-YEARS

0.5

## PROFESSIONAL

0.5

## OTHER

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We Wish to elucidate conformations of DNA in solution, and to investigate their relationships to genetic function, interactions with drugs, and protein recognition. The method we have chosen to carry out such studies is nuclear magnetic resonance (NMR) spectroscopy; more specifically the method used to detail these solution conformations is the 2D-NOE NMR method, which provides relative interatomic distances.

Loops in DNA are expected to be important for protein recognition of selective genetic sites, such as RNA polymerase promotor sequences. Consequently, we have previously studied quasipalindromic synthetic oligonucleotides and the mechanism of hairpin loop formation.

In addition to studies with normal oligos, we are investigating phosphorothioate oligo duplexes by the above NMR methods. This is complicated by the fact that due to non-stereospecific synthesis each thio-phosphate can exist in two stereoisomeric forms. 2D-NOE NMR with molecular modeling is being used to characterize the conformations(s) of the duplex form of the oligo analogs, by comparison with the normal oligo of the same base sequence.

## Major Findings

### 2DNOE NMR Studies of Phosphorothioate Oligodeoxynucleotide Duplexes

We have synthesized sufficient quantities of the self-complementary Dickerson dodecamer d-CGCGAATTCGCG as both the normal and S-oligo analog. We are subjecting solutions of these compounds to 2D NMR analysis, using several pulse sequences. By comparison with the normal oligo, which is known to adopt a B-form DNA conformation, we expect to be able to establish the detailed conformation of the S-analog duplex.

### Molecular Graphics and Conformational Calculations

We have an Evans & Sutherland PS390 molecular graphics system interfaced with a DEC micro-VAX-II mini computer. We have also had access to a Silicon Graphics Iris data-station in DCRT. We carried out extensive testing of several programs: MOGLI, FRODO, QUANTA, SYBYL, and BIOGRAF. We chose BIOGRAF as the best program because it is most convenient for use with DNA, and has the most convenient interface for energy minimization and molecular dynamics. We have used this system to investigate the conformations of duplexes of the S-oligo analog, and with groups linked to DNA duplexes. We have also begun a collaboration with the Advanced Scientific Computer Laboratory in Frederick in order to be able to generalize conclusions regarding the conformations of a range of chemically modified oligonucleotide analogs. These calculations should be a valuable guide to selecting appropriate analogs for synthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06523 05 M

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism, Irreversible Binding and Mechanism of Action of Etoposide (VP-16,213)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Birandra K. Sinha, Ph.D.	Principal Investigator	MB, COP, DCT, NCI
Pedro Politi, M.D.	Visiting Fellow	MB, COP, DCT, NCI
Helen M. Eliot	Biologist	MB, COP, DCT, NCI

COOPERATING UNITS (if any)

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LAB/BRANCH

Medicine Branch

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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS

0.5

PROFESSIONAL:

0.5

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

VP-16 undergoes O-demethylation to generate active intermediates that binds to protein and DNA. The O-demethylation is P450 dependant. Peroxidases, such as horseradish or prostaglandin synthetase, also activate VP-16 and VM-26 to their O-Quinone derivatives, and catalyze binding of reactive intermediates to DNA. We have shown that both the dihydroxy and O-Quinone derivatives are cytotoxic and induce topo II-dependent cleavage. The binding sites on topo-II-DNA complex for these O-demethylated drugs are similar to the parent compound. Thus, enzymatic activation to reactive intermediates is important in the biological activities of VP-16 and VM-26.

## Major Findings

The semisynthetic podophyllotoxin derivative etoposide (VP-16) has shown activity against a number of human tumors. Although the mechanism of action of this drug is not clear, DNA damage induced by VP-16 has been suggested for its cytotoxicity. Recently, we have proposed that the cellular damage induced by VP-16 may result from the formation of a reactive intermediate during bioactivation of the drug. We have studied the metabolism of VP-16 by mouse hepatic microsomes. Using HPLC analysis of the chloroform extracts of the microsomal incubation it was shown that VP-16 formed the 3'-4'-dihydroxyl derivative (DHVP). The formation of this metabolite (2% of the parent drug) was NADPH-protein-VP-16 and time dependent suggesting that the activation was enzymatic. Moreover, DHVP formation was inhibited by SKF-525A and piperonylbutoxide suggesting that the O-demethylation was P-450 dependent.

Incubation of [ $^3\text{H}$ ] VP-16 with microsomes containing NADPH and DNA resulted in irreversible binding of the drug to DNA and proteins.

We have found that peroxidase catalyzed activation of VP-16 forms a number of reactive metabolites. HPLC and mass spectral analysis have shown that VP-16 undergoes aromatization (to Ar-VP-16-Q) which is subsequently O-demethylated to O-Quinone (Ar-VP-16-Q). Inhibition studies suggest that the protein binding species result from O-demethylation reactions (VP-16-Q and AR-VP-16-Q) and that DNA binding species are positively charged.

Using alkaline elution studies in a sensitive and resistant MCF-7 cells recently, we have found that VP-16 induces a significant amount of DNA damage in the sensitive cells. In contrast, very little DNA damage could be detected in the resistant cells. Furthermore, when isolated nuclei were used to assess DNA damage, there was only two-fold difference in VP-16 induced DNA strand breaks between the sensitive and resistant cells. The differences in toxicity (~200 fold), and uptake of VP-16 (2-3-fold) do not completely explain DNA damage induced by VP-16 in these cells and suggest that other factors may also be involved in the mechanics of cell kill by VP-16.

Resistance to VP-16 and other antitumor drugs results in a decreased drug accumulation and in multidrug resistant cell lines overexpression of P-170 glycoprotein has been implicated in this decreased drug accumulation. In order to characterize VP-16 resistance, we have carried out uptake and efflux of VP-16 in a number of sensitive and resistant human tumor cell lines. Our studies suggest that decreased VP-16 accumulation is not due to overexpression of the P-170 glycoprotein, but it may be related to energy-dependent modification in drug binding in the resistant cell lines.

Further, using photoaffinity labelling of P-170 protein with photoactive vinblastin and verapamil analogs, we have recently shown that VP-16 has very low binding affinity for the protein, indicating that P-170 is not the major mechanism for VP-16 resistance. Recent studies also indicate that a VP-16 metabolite, dihydroxy VP-16, formed from O-demethylation of VP-16 chelates metal ions and in the presence of  $\text{H}_2\text{O}_2$  or reduced glutathione forms hydroxyl radicals which induce topo-II independent DNA cleavage. We have shown that O-demethylated compounds bind to topo II and the binding is similar to VP-16, and induce DNA cleavage.

PUBLICATIONS

Sinha BK, Eliot HM, Kalyanaraman B. Non-dependent hydroxyl radical formation and DNA damage from a novel metabolite of the clinically active antitumor drug, VP-16. FEBS Lett 1988;227:240-4.

Sinha BK, Haim N, Dusre L, Kerrigan D, Pommier Y. DNA strand breaks produced by etoposide (VP-16, 213) in the sensitive and resistant human breast tumor cells: implications for the mechanism of action. Cancer Res 1988;48:5096-100.

Sinha BK. Role of free radicals in etoposide (VP-16, 213) action. In: Simic MG, Taylor KA, von Sonntag C, eds. Oxygen Radicals in Biology and Medicine, New York: Plenum Press 1989;765-68.

Politi P, Sinha BK. Role of differential uptake, efflux, and binding of etoposide in sensitive and resistant human tumor cell lines: implications for the mechanisms of drug resistance. Mol Pharm 1989;35: 271-8.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06524 03 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inhibition of Gene Expression by Oligodeoxynucleotide Analogs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Jack S. Cohen, Ph.D.	Principal Investigator	MB, COP, DCT, NCI
Christine Subasinghe, B.A.	NCI Fellow	MB, COP, DCT, NCI
Kenya Mori, Ph.D.	Visiting Fellow	MB, COP, DCT, NCI
Jerry Jaroszewski, Ph.D.	Visiting Associate	MB, COP, DCT, NCI
C.A. Stein, M.D.	Clinical Associate	MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

S. Broder, M. Matsukura, and H. Mitsuya, COP, DCT; G. Zon, Applied Biosystems Inc.; L. Neckers, MB; S. Wilson LB, NCI; J. Dahlberg, Pan-Data; Y.C. Cheng, UNC; J. Reed, Univ. Penn; D. Kuchington, St. Mary's Hospital, London; K. Molling, Max Planck Inst., W. Berlin; J.J. Toulume, et. al, Paris

## LAB/BRANCH

Medicine Branch

## SECTION

Biophysical Pharmacology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.0

## PROFESSIONAL

3.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Normal oligodeoxynucleotides (ODNs) have been reported to have inhibitory effects on selected gene expression, primarily due to transcription arrest by duplex formation with mRNA. However, these compounds are susceptible to digestion by cell nucleases. Nuclease-resistant ODN analogues containing methyl phosphonate (P-Me) in place of phosphate has been developed to overcome this problem, but these compounds also have disadvantages, notably poor aqueous solubility and poor hybridizability. In order to overcome these difficulties, we have synthesized a series of phosphorothioate (P-S) analogs, which retain the charge of phosphate, but are nuclease-resistant. These compounds have been tested for inhibition of protein expression in cell free systems, and as antiviral agents against HIV and other lentiviruses, against herpes simplex virus (a DNA virus), and several mammalian genes, including oncogenes (eg. c-myc), and against the gene related to drug resistance (mdr-1). Tests for toxicity in mice are also being conducted by DTP, NCI. A series of analogs with covalently attached groups on the 5' end of oligomers are being synthesized. These have been used to monitor cellular uptake (using the fluorescent acridine group), and they are also being tested for biological activity.

### Major Findings

During the past year, several book chapters were written on this subject, a book was edited J.S. Cohen to be published by Macmillan in the UK, and a conference was organized by a Committee chaired by J.S. Cohen, that was co-sponsored by NCI and NIAID, with over 300 attendees

### Phosphorothioate Oligonucleotides are Potent Sequence Non-specific Inhibitors of de novo Infection by HIV (In collaboration with Dr. M. Matsukura)

Phosphorothioate homo-oligonucleotides have been shown to protect ATH8 cells against the cytopathic effect of de novo infection by HIV (Matsukura et. al., PNAS 84: 7706, 1987). The effect is dose and chain-length dependent, with a maximum effect seen for 21-28 mers. We have now synthesized a series of phosphorothioate oligomers with mixed-base sequences and found that all of them have a dose dependent cytoprotective effect which is maximal at an oligomer concentration of ca. 1-2  $\mu\text{M}$ . The least effective sequences contain only A or T and the most effective studies have 40% GC content or greater. The results also confirm the length effect, namely that 21 mers are more cytoprotective than 14 mers.

### Phosphorothioate Oligodeoxynucleotides Specifically Inhibit the Replication of Lentiviruses (In collaboration with Dr. J. Dahlberg)

Phosphorothioate analogs of oligodeoxynucleotides are potent inhibitors of the cytopathogenicity of animal lentiviruses. For example, several such oligonucleotides with different base sequences at 2  $\mu\text{M}$  concentration protected Tahr cells by a factor of  $10^5$  against caprine arthritis and encephalitis (CAEV) and equine infectious anemia virus (EIAV). The characteristics of this inhibition, in terms of the absence of base-sequence and dependence on oligonucleotide length, are similar to that previously described for inhibition of HIV in ATH8 cells (Matsukura, et.al., Proc. Natl. Acad. Sci. USA 84: 7706, 1987). Thus, the 28-mer homo-oligomer of cytidine (S-dC<sub>28</sub>) was at least effective as three antisense sequences targeted on the LTR, *gag*, and *env* regions of CAEV. The effectiveness of homo-oligomers was in the order C>A>T, and a random 28-copolymer with the composition 2C:1G was at least as effective as S-dC<sub>28</sub>. The order of length effectiveness was 28>14>5 for all base compositions tested. By contrast, these compounds did not inhibit the cytopathogenicity of two type C retroviruses, murine leukemia (MuLV) and baboon endogenous virus (BEV). One major difference between these classes of retroviruses is the fact that the lentiviruses have reverse transcriptases (RT) that require Mn<sup>2+</sup>-dependent RT. We surmise that the mechanism of action of these compounds may involve an inhibition of the polymerase function of RT.

### Synthesis and Properties of Novel 5'-Linked Oligos

We have synthesized oligos with 5'-linked acridines in the automated synthesizer using and intermediate acridine-linked phosphoramidite. With the acridine moiety linked via the 9-amino position, 6-chloro-2-methoxyacridine resulted in facile substitution by thiophenol at the 6 position. Using a non-substituted acridine with dA, as

observed previously by Helene and co-workers. However, use of blocked A, G, and C monomers resulted in base-catalyzed cleavage of the linker at the 9-amino acridine position. In order to overcome this problem, we used an alternative synthesis with a maleimide linked acridine (Nara & Tuzimura, *Agric. Biol. Chem.*, 42: 793, 1978), which was attached to a mercaptopropanol linker (Connolly and Rider, *Nucl. Acids Res.* 13: 4485, 1985). In this way we were able to prepare linked acridine with any sequence oligo. We have also prepared anthroquinone-linked oligomer using a linked phosphamide that is not sensitive to alkaline cleavage.

#### Uptake of Oligos into Cells (In collaboration with Dr. L. Neckers)

When small antisense oligodeoxynucleotides (ODNs) complementary to the 5' region of *c-myc* mRNA are added to intact cells in culture, *c-myc* protein synthesis is specifically inhibited. Furthermore, addition of specific antisense ODNs to culture medium inhibits intracellular viral replication. These data suggest that unsubstituted ODNs can penetrate cells. However, such findings contrast with the prevailing view that cells are impermeable to ODNs. In order to design more effective synthetic antisense ODNs as potential clinical agents, we synthesized oligo dT<sub>n</sub> compounds containing 5'-linked acridine and utilized these fluorescent derivatives, in conjunction with flow cytometric analysis, to examine the mechanism of cellular uptake. We found that these compounds, up to 20 bases in length, are taken up by cells in a saturable and size-dependent manner compatible with a receptor mediated endocytotic process. Although neither nucleosides nor deoxyribose 5' phosphate are competitors of uptake, polynucleotides of any length will competitively inhibit ODN transport, providing they possess a 5' phosphate moiety. Using oligo dT-cellulose as a tool for affinity purification, we have identified an 80 kD cell surface protein which may mediate ODN transport.

#### Inhibition of Protein Expression by Phosphorothioate Oligonucleotides in Cell-Free Systems (In collaboration with Dr. J.J. Toulmè, et al)

We have studied the translation of rabbit globin mRNA in cell free systems (reticulocyte lysate and wheat germ extract) and in microinjected *Xenopus* oocytes in the presence of anti-sense oligonucleotides. Results obtained with the unmodified all-oxygen compounds were compared with those obtained when phosphorothioate or  $\alpha$ -DNA was used. In the wheat germ system, a 17-mer sequence targeted to the coding region of  $\beta$ -globin mRNA was specifically inhibitory when either the unmodified phosphodiester oligonucleotide or its phosphorothioate analog were used. In contrast, no effect was observed with the  $\alpha$ -oligomer. These results were ascribed to the fact that phosphorothioate oligomers were shown to elicit RNase-H activity comparable to the all-oxygen congeners, while the  $\alpha$ -DNA/mRNA hybrids were a poor substrate. Microinjected *Xenopus* oocytes followed a similar pattern. The phosphorothioate oligomer was more efficient to prevent translation than the unmodified 17-mer. Inhibition of  $\beta$ -globin synthesis was observed in the nanomolar concentration range. This result can be ascribed to the nuclease resistance of phosphorothioates as compared to phosphodiester linkages.  $\alpha$ -oligomers were devoid of any inhibitory effect up to 30  $\mu$ M. In both cell-free systems and oocytes, phosphorothioate oligodeoxynucleotides were shown to be non-specific of protein translation at concentrations in the micro-

molar range. Non-specific inhibition of translation is dependent on the length of the phosphorothioate oligomer. These non-specific effects were not observed with the unmodified or the  $\alpha$ -oligonucleotides.

#### Regulation of the *mdr-1* Gene in MCF-7 Cells with Phosphorothioate Analogs of Oligonucleotides

The ability of malignant cells to develop resistance to chemotherapy is one of the major obstacles in cancer treatment. One major change that occurs during transmission of wild type to drug-resistant cancer cells is over expression and/or amplification of the *mdr-1* gene, that codes for a membrane glycoprotein, P170. This is believed to function as an efflux pump for xenobiotics entering the cells, thus rendering the cells resistant to a variety of drugs, producing multiple drug resistance (*mdr*). We have attempted to reverse drug resistance in human breast carcinoma cells by exposing them to phosphorothioate oligodeoxynucleotide (S-oligo) analogs with antisense sequences to regions of the *mdr-1* gene. Incubation of the cells with  $^{35}\text{S}$ -labelled oligos resulted in incorporation of the radioactivity mainly into the cytoplasm. Exposure of the cells to antisense, sense and random sequences of S-oligos affected cell growth and their vulnerability to adriamycin toxicity in a sequence-dependent manner. Effects of oligo length, concentration, and site of sequence are being studied.

#### Inhibition of HIV-1 Reverse Transcriptase and RNase-H *In Vitro* by Phosphorothioate Analogs of Oligonucleotides (In collaboration with Dr. K. Molling)

Nuclease-resistant phosphorothioate analogs of certain oligodeoxynucleotides (S-oligos) were tested *in vitro* with purified recombinant HIV-1 reverse transcriptase (RT) and RNase-H. It was shown previously that the HIV-1 codes for a polyprotein p66 containing domains for both these activities (Hansen et. al., Embo J. 7: 239, 1988). Synthetic poly(rA)-oligo(dT) was used as a substrate for the RT, and radioactively labelled M13 DNA was transcribed as a substrate for RNase H. The RNase-H was free of cellular RNase of *E. coli* origin. A 28-mer S-oligo (S-dC<sub>28</sub>) exhibited the most potent inhibitory effect on RTm with an ID<sub>50</sub> of 0.1 ng/ml. These data confirm recently published observations of Majumdar et.al., (Biochemistry 28: 1340, 1989). A 21-mer phosphorothioate was slightly less inhibitory, a 14-mer 100-fold less, therefore showing a length dependence. Normal oligos did not significantly inhibit the RT. A 28-mer of oligodeoxyadenosine (S-dA<sub>28</sub>) also inhibited the RT, whereas the normal and S-analogs of oligothymidine did not. This indicated some base-composition specificity of the RT *in vitro*. The RNase-H was inhibited by S-dC<sub>21</sub>, SdC<sub>28</sub>, and S-dA<sub>28</sub> to a similar degree as RT. By contrast, most dramatic inhibition of the RNase-H was observed with S-S-dT<sub>28</sub>, with an ID<sub>50</sub> of 10 ng/ml. This compound appears RNase-H specific since it has no effect on the RT. Its 100-fold stronger effects of the RNase-H than on the RT suggests that may be possible to design inhibitors specific for the RNase domain of p66. The results for the RT polymerase interaction are consistent with the findings of Matsukara et.al. (PNAS 84: 7706, 1987), that SdC<sub>28</sub> is a more potent inhibitor of the cytopathic effects of HIV-1 *in vitro* than are S-dA<sub>28</sub> or SdT<sub>28</sub>.

Gag and Pol Antisense Oligodeoxynucleotides as Inhibitors of HIV-1 (In collaboration with Dr. D. Kinchington)

Sequences from the *gag-pol* region of the HTLV-III RF strain of HIV-1 were chosen as targets for antisense oligodeoxynucleotides, both normal (O-oligos) and phosphorothioate analogs (S-oligos). They were: overlapping sequences from the splice site for the P-16/P24 transcript, the *gag-pol* frame-shift sequence, the junction between the protease and the RT transcript, and a sequence taken at random from within the polymerase reading frame. Seven antisense sequences ranging from 13 to 24 deoxynucleotides in length were synthesized and were tested in a *de novo* infection assay with the C8166 T-lymphocyte cell line infected with HTLV-III RF strain. Oligos were tested in log dilutions from 0.01 to 10  $\mu$ M. The S-oligos all exhibited an  $ED_{50}$  in the 1-10  $\mu$ M range, while the O-oligos were inactive up to 10  $\mu$ M. The data obtained to date shows that S-oligos to a P18/P24 sequence (22-mer), the frame-shift sequence (24-mer) and the protease active site sequence (22-mer) were somewhat more active in this assay system than the other four compounds, which were shorter in length (13-21 mers). These results are in agreement with previous *in vitro* studies (Matsakura et.al., Proc. Natl. Acad. USA 84: 7706, 1987) which show that although S-oligos are active against HIV in *de novo* infected cells, the activity is not sequence dependent. Further studies are being carried out to test these *gag-pol* S-oligos in chronically infected cells.

Effect of Phosphorothioate Oligodeoxynucleotides on HSV DNA Polymerase (In collaboration with Dr. Y.C. Cheng)

Herpes simplex virus (HSV), a DNA virus, has been associated with a wide spectrum of clinical diseases, including certain types of cancer. A viral type-specific DNA polymerase, which is different from the host enzymes, is induced in host cells upon HSV infection. This viral polymerase plays an essential role in viral replication and is considered a prime target for development of antiviral compounds. One approach is to develop anti-template compounds that inhibit viral DNA synthesis.

Effects of phosphorothioate oligodeoxynucleotides of different chain length and base composition on herpes simplex virus (HSV) type 2 (strain 333) induced DNA polymerase have been examined *in vitro*. The anti-HSV-2 DNA polymerase activity was related to the base composition of the analogs, with the order of potency: deoxycytidine > thymidine > deoxyadenosine, for compounds with equal chain length. The potency was also related to oligomer chain length, since it was observed that the longer the chain length, the more potent the inhibition exerted. Among all the compounds tested, the phosphorothioate oligodeoxycytidine 28-mer (S-(dC)<sub>28</sub>) was the most potent inhibitor of HSV-2 induced DNA polymerase. This inhibition was competitive with an activated DNA template with a  $K_i$  value of 7 nM. It was also a competitive inhibitor of the DNA polymerase associated exonuclease activity with a  $K_i$  value of 5 nM. In contrast, this compound showed less inhibition of human DNA polymerase  $\alpha$ ,  $\beta$ , and  $\gamma$ , as well as HSV-1 (strain KOS) and Epstein-Barr Virus induced DNA polymerase. The possibility that S-oligomers can serve as primers for DNA elongation was also investigated. Poly(dG) · S-(dC)<sub>28</sub> and poly(dA) · S-(T)<sub>28</sub> are poor substrates for DNA elongation catalyzed by DNA polymerase. In summary, phosphorothioate oligonucleotides could be an anti-template inhibitor of HSV DNA polymerase. This information may lead to the development of a new class of selective anti-HSV agents.

Comparison of Normal and Phosphorothioate Antisense *bcl-2* Oligodeoxynucleotides on Cellular Proliferation (In collaboration with Dr. J. Reed)

Antisense oligodeoxynucleotides specific for sequences in mRNAs from the B cell lymphoma/leukemia-2 (*bcl-2*) gene were used to inhibit the growth of human lymphoma and leukemia cell lines. Normal and phosphorothioate oligodeoxynucleotides were compared with regard to, specificity, potency, and kinetics. The latter compounds are extremely nuclease-resistant, making them candidates for eventual use *in vivo*. Both normal and phosphorothioate antisense *bcl-2* oligodeoxynucleotides were specific inhibitors of cellular proliferation, since sense versions of these oligodeoxynucleotides were inactive at similar concentration (up to 250  $\mu$ M for normal and 25  $\mu$ M for phosphorothioates). Phosphorothioates were more potent, however, with half-maximal inhibition of lymphoma and leukemia cell growth occurring at concentrations of approximately 15-25  $\mu$ M in serum-free cultures, as opposed to approximately 125-250  $\mu$ M for normal oligodeoxynucleotides. With normal oligodeoxynucleotides, inhibition was detectable within one day of addition of cultures, whereas phosphorothioates were ineffective until 3-4 days, consistent with recent investigations of the kinetics of the kinetics of normal and phosphorothioate oligodeoxynucleotide entry into cells. Inhibition by antisense oligodeoxynucleotides was not attributable to mere formation of oligodeoxynucleotide-RNA duplexes in cells, suggesting specific ablation of *bcl-2* expression through a sequence-dependent mechanism. Taken together, these data indicate that normal and phosphorothioate oligodeoxynucleotides targeted against human oncogenes can be specific inhibitors of cellular proliferation, and provide indirect evidence that the *bcl-2* gene plays an important role in regulating the growth of normal and neoplastic lymphocytes.

PUBLICATIONS

Stein CA, Mori K, Loke SL, Subasinghe C, Shinozuka K., Cohen JS, Neckers, LM. Phosphorothioate oligodeoxynucleotides with 5'-linked acridine: characterization and preliminary kinetics of cellular uptake. *Gene* 1989;72:333.

Loke SL, Zhang XM, Stein CA, Avigan M, Cohen JS, Neckers LM. Delivery of c-myc antisense phosphorothioate oligodeoxynucleotides to hematopoietic cells in culture by liposome fusion. *Curr Top Microbiol Immunol* 1988;141:282.

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Stein CA, Cohen JS. Oligodeoxynucleotides as inhibitors of gene expression: a review. *Cancer Res* 1988;48:870.

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Mori K, Subasinghe C, Cohen, JS. Oligodeoxynucleotides analogs with 5'-linked anthraquinone. *FEBS Lett*, in press.

Cazanave L, Stein CA, Loreau N, Thuong NT, Neckers LM, Subasinghe C, Helene, Cohen JS, Toulme JJ. Comparative inhibition of rabbit globin mRNA translation by modified antisense oligodeoxynucleotides. *Nucl Acids Res*, in press.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06716 02 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Platinum drug resistance in human malignancies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Eddie Reed, M.D.	Principal Investigator	MB, COP, DCT, NCI
Ricardo Parker, Ph.D.	Biotechnology Fellow	MB, COP, DCT, NCI
Freida Bostick-Burton, B.S.	Biologist	MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

Laboratory of Cellular Carcinogenesis and Tumor Production, DCE, NCI; Laboratory of Molecular Pharmacology, DTP, DCT, NCI; University of Southern California, Los Angeles, California

## LAB/BRANCH

Medicine Branch

## SECTION

Experimental Therapeutics Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.0

## PROFESSIONAL:

2.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The Principal Investigator has shown that platinum drug resistance can be studied in cancer patients actively receiving chemotherapy. This laboratory activity is therefore one that seeks to bridge basic science and clinical activities using fresh human tissues whenever possible. The focus is on drug resistance in the cancer patient. Briefly, several different, but related observations have been made.



Major Findings

1. In a cohort of ovarian cancer patients receiving single agent cisplatin or carboplatin therapy, the level of platinum-DNA adduct in leukocyte DNA is more closely related to disease than any previously identified prognostic variable in that disease. In a collaborative study with the University of Southern California, preliminary data suggest that adduct level in leukocyte DNA measured by atomic absorbance spectroscopy on cycle one of the therapy may be sufficient to determine who will and who will not respond. In addition, autopsy data shows that adduct levels in bone marrow are consistently similar to adduct levels in tumor tissues. Collectively, these data (along with previously published work) suggest that pharmacogenetics may be an important component of cancer drug resistance.
2. A human excision nuclease, ERCC1, has been identified which confers resistance to cisplatin in UV-repair deficient CHO cells of complementation group I. ERCC1 is expressed to some degree in every human ovarian and colon tumor cell line studied thus far, and is expressed in RNA isolated from isolated peripheral blood leukocytes. Preliminary data suggests that the level of expression of the gene may be related to the level of resistance to cisplatin, and that this gene may contribute to clinical platinum drug resistance.
3. Method development for an assay to assess functional platinum-DNA adduct repair capability in human cells has progressed to the point where we will begin to adapt this methodology to fresh human tissues. This method involves platinating the plasmid pRS-Vcat to a defined level, and then transfecting the cisplatin-modified plasmid cells and quantitating the ability of the cell to repair the plasmid DNA.

PUBLICATIONS

- Reed E, Ozols RF, Tarone R, Yuspa SH, Poirier, MC. The measurement of cisplatin-DNA adduct levels in testicular cancer patients. *Carcinogenesis* 1989;9:1909-11.
- Litterst CL, Reed E. Platinum compounds. In Kaiser HE, ed. *Progressive stages of malignant neoplastic growth*. London: Alden Press, 1989;85-97.
- Reed E, Kohn KW. Cisplatin and platinum analogs. In: Chabner BA, ed. *Cancer chemotherapy--principles and practice*. Philadelphia: J.B. Lippincott, 1989, in press.
- Poirier MC, Liou S, Reed E, Strickland PT, Tockman MS. Determination of carcinogen-DNA adducts by immunoassay. *J of UOEH* 1989;11(suppl):353-67.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06717 01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Genetic and biochemical differences of glucose metabolism in breast cancer cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Charles E. Myers, M.D.	Chief, Medicine Branch	COP, DCT, NCI
Grace Wan-pin Chao Yeh, Ph.D.	Senior Investigator	MB, COP, DCT, NCI
Carlos Jamis-Dow, M.D.	Visiting Fellow	MB, COP, DCT, NCI
Sandy Occhipinti	Biologist	MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

## LAB/BRANCH

Medicine Branch

## SECTION

Experimental Therapeutics Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.0

## PROFESSIONAL

2.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Adriamycin, one of the anthracycline antibiotics, is an active chemotherapeutic agent against a wide range of human neoplasms and is especially effective against solid tumors such as carcinoma of the breast, lung, thyroid, ovary, and soft tissue sarcomas. Although adriamycin (Adr) and daunomycin are two of the most active anticancer drugs, there is no clear consensus as to their cytotoxicity mechanisms. Both drugs undergo oxidation and reduction in NADPH-dependent reactions to generate superoxide, hydrogen peroxide and hydroxyl radical, drug radicals and drug quinone methods all potentially cytotoxic in tumor cells. Both Adr activation and detoxification mechanisms are coupled with NADPH required reactions. The formation of NADPH primarily depends on the pentose phosphate shunt and an increase in the activity of this pathway is a commonly used index of cellular oxidant stress.

## Major Findings

We have shown that a cloned line of human breast tumor cells, MCF-7 with pleiotropic drug resistance, ADR<sup>R</sup>, exhibited marked alterations in the response of pentose shunt activity to exogenous peroxides as compared to the parent cell line, MCF-7 wild type (WT). Importantly, ADR<sup>R</sup> had only 1.3% of the glucose-6-phosphate dehydrogenase (G6PD) activity in the WT cells. The decreased G6PD, the key regulatory enzyme of pentose shunt, activity in ADR<sup>R</sup> suggests that the formation of Adr free radicals decreased by ADR<sup>R</sup> by decreasing the availability of NADPH. In order to test this hypothesis and to see if a change is an early event in the development of the resistant phenotype we have developed other independently derived Adr resistant cell lines. New Adr resistant cells were developed from the sensitive MCF-7 (Adr IC<sub>50</sub> =  $4.06 \pm 0.13$  nM) by exposure to increasing concentration of drug. We examined the biological changes on our newly derived cells at early intermediate and late stages of Adr resistance, AR10, AR60, AR200, AR250, AR400, and AR600, that are 12, 63, 241, 250, 425, and 585-fold more resistant to Adr than the WT cells, respectively. The resistance to Adr in our newly developed cells has been stable over a period of 50 passages. Although the cells were selected by exposure to Adr, they also show cross resistance to vinblastin, one of the drugs associated with the multidrug resistant phenotype. Therefore our newly pleiotropic drug resistance. We measured G6PD, glutathione peroxide, total glutathione-S-transferase (GST) and anionic glutathione-S-transferase (GST  $\pi$ ) activities in our multidrug resistant cells. Total GST were increased moderately (2-fold) in AR200 and AR250, but no GST activity was detected in any of our resistant cells. Glutathione peroxidase was measured in the cells using either cumene hydroperoxide or hydrogen peroxide as substrate to differentiate between organic and inorganic peroxidase activities. There are differences in peroxidase activities among the different stages of resistance but in each stage similar results were obtained with either peroxide as substrate. This also supports our data that GST  $\pi$  has intrinsic organic peroxidase activity. The major differences between our multidrug resistant cells and the WT is in their levels of G6PD activity. We find a substantial decrease in G6PD activity in all the resistant cells. A significant decrease in G6PD activity was observed even at the earliest stage of resistance, cells 12 times more resistant than the WT cells. G6PD activities for the WT, AR10, AR60, AR200, AR250, AR400, and AR600 cells are  $735 \pm 44.5$ ,  $332.6 \pm 10.4$ ,  $313.1 \pm 33.3$ ,  $257 \pm 10.2$ ,  $182.3 \pm 17.5$ ,  $165.2 \pm 33$ , and  $110.9 \pm 14.5$  nmole/min/mg protein. (mean  $\pm$  SE), respectively. The G6PD activities show an inverse logarithmic relationship with Adr IC<sub>50</sub> with a high degree of correlation. We further examined the G6PD kinetics in the WT cells and in cells at the earliest stage of resistance. The K<sub>m</sub> for NADP<sup>+</sup> was similar in both cells. Therefore, we conclude that early events in G6PD activity changes in V<sub>max</sub> not in K<sub>m</sub>. We also found our new drug resistant cells overexpressed the gp-170 membrane protein. The level of gp-170 expression in the WT is negligible (0.0026 A.U.) A 10-fold increase from the AR10 cells to the AR250 cells was observed and a linear correlation between gp-170 expression and the degree of Adr resistance was found. We further examined the glucose metabolism through glycolysis pathway in WT and Adr resistant cells. We found a 75-fold increase in lactate production in ADR<sup>R</sup> than WT cells and also a markedly increase in our newly developed Adr resistant cells. The changes of glucose flux to lactate in Adr resistant cells increased the production of ATP through the glycolysis pathway. The increased ATP generation in ADR<sup>R</sup> may be essential for the function of gp-170. The relative levels of gp-170 expression in the cells correlate well with their Adr IC<sub>50</sub> and also correlate with their lactate production rates. Since gp-170 is an ATPase, these results suggest

that cells with increased gp-170 may have higher lactate production rates that reflect stimulation of their anaerobic glycolysis pathway due to increased ATP utilization. We are studying the G6PD expression at the molecular level in our laboratory. Total RNA was extracted by the guanidinium/cesium chloride method and hybridized against a human G6PD cDNA probe. We found the RNA expression of G6PD in Adr resistant cells was markedly decreased in AR200, AR250, AR400, and AR600 cells and a 10-20 fold decrease in G6PD message was observed in our newly cloned Adr resistant MCF-7 cells at different stages of drug resistance. Therefore, we hypothesize that G6PD may be important for Adr activity and gp-170 may be important for Adr efflux. Both changes certainly complement each other in Adr detoxification. Whether this regulation is at the gene expression and/or amplification levels is under our current investigation.

#### PUBLICATIONS

Yeh GC and Phang JM. Stimulation of the phosphoribosyl pyrophosphate and purine nucleotide production by pyrroline 5-carboxylate in human erythrocytes. *J Biol Chem* 1989;263:13083-9.

Merrill MJ, Yeh GC, Phang J Purified human erythrocyte pyrroline-5-carboxylate reductase. *J Biol Chem* 1989;264:9352-8.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06718-01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

Human folate binding/transport proteins (FBPs)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)

Patrick Elwood, M.D., Ph.D.

Principal Investigator

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Clement Knight, M.D.

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## COOPERATING UNITS (if any)

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## LAB/BRANCH

Medicine Branch

## SECTION

Molecular Cell Biology Section

## INSTITUTE AND LOCATION

NCI, NIH Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The Molecular Cell Biology Section has investigated:

1. The structure, function, and molecular biology of human folate binding/transport proteins.
2. The role of these FBPs in the transport of folates and folate analogues and in the development of methotrexate resistance in vitro.
3. The expression of these FBPs.

## MAJOR FINDINGS

### 1. The structure, function, and molecular biology of human FBPs

We have previously isolated and characterized human FBPs, a membrane-associated FBP (M-FBP) contained in the membrane fractions of tissues and a soluble FBP (S-FBP), from human placenta, milk, and malignant tissue culture cells (KB cells). The transport of physiologic folates and methotrexate (Mtx) under physiologic conditions (relative to folate concentration) is inhibited by  $\geq 85\%$  with antisera to these FBPs demonstrating an important role of these glycoproteins in folate/Mtx transport. To study the primary structure of FBPs, we have determined the partial internal amino acid sequence of cyanogen bromide fragments of both the soluble and membrane forms of FBPs. Human placental and KB cell cDNA libraries were screened with oligonucleotide probes deduced from these amino acid sequences and multiple clones were isolated. Characterization of these cDNA clones has demonstrated that: 1) the human soluble and membrane-associated forms of FBPs are single gene products encoded for by an 1100 bp species of mRNA; 2) the mature M-FBP contains 257 amino acids (calculated MW = 29,817, and pI = 9.6), 3 potential N-linked glycosylation sites, and multiple potential phosphorylation sites including a consensus cyclic AMP protein kinase site; 3) the FBPs are extensively glycosylated such that approximately 29% of the apparent  $M_r$  on SDS-PAGE analysis is due to carbohydrates; 4) the FBPs are variably expressed in human tissues (placenta > epithelium > brain >> liver); 5) the FBPs contain numerous (17) conserved cysteines and tyrosines which, together with several clusters of charged amino acids, are most likely important in secondary/tertiary structures and ligand binding; and 6) the FBP has an amino terminal sequence consistent with a leader peptide that is 25 amino acids long and a carboxyl terminus which appears to serve as a membrane anchor. To further investigate the FBP gene, the cDNA clones have been used to screen a human leukocyte genomic library and to localize gene on a specific chromosome. Characterization of these genomic clones should further elucidate the structure and regulation of these important transport proteins. Preliminary Southern blot analysis of genomic DNA derived from CHO hybridomas containing known human chromosomes suggest that several genes for FBPs are contained on human chromosome #11 and that the gene exists in at least two allelic forms.

We are using compute modeling of the amino acid sequence to attempt to predict important functional domains (e.g. ligand binding sites, transmembrane domains, or epitopes). Construction of deletion constructs of the FBP cDNA followed by transfection, should also allow us to better define important functional domains.

Since the human domains are involved in transport and since other similar transports are phosphorylated, phosphorylation of M-FBP has been studied in vitro and in intact tissue culture cells. Preliminary data indicates that M-FBP is phosphorylated by protein kinase C in vitro and in vivo. Although the physiologic significance of this post-translation modification remains unclear. It is likely to be involved in ligand binding and/or translocation of M-FBP across the plasma membrane.

## 2. The role of M-FBP in Mtx transport and resistance.

Human malignant nasopharyngeal epidermoid carcinoma (KB) cells provide a good model for these studies since expression of M-FBP is easily detected in the wild type cell line and since we have previously demonstrated an essential role for this protein in these cells for Mtx transport. We have isolated numerous Mtx clones from KB cells following exposure to 10  $\mu$ M Mtx or following exposure to increasing doses of Mtx (10 nM increased slowing to 150 nM) cultured in folate replete and folate deplete (physiologic depletions) media. Preliminary results indicate that the expression of M-FBP is commonly associated with the development of Mtx resistance and is correlated with alteration of the transport of Mtx. Studies are underway to further characterize these malignant Mtx-resistant clones.

## 3. Regulation of expression of FBPs.

We have shown that the expression of FBPs are related to the intra- and extra-cellular folate concentration in tissue culture. Similar experiments are planned using nucleic acid probes and Northern blot analysis to study the effect of changes in folate concentration And the effect of Mtx exposure in tissue culture cells and to determine the time course of changes in mRNA expression. Characterization of the genomic clones, including sequence analysis and transfection experiments, should allow isolation of regulatory elements of the gene and further elucidation of factors involved in the regulation at a transcriptional level.

## PUBLICATIONS

Elwood PC. Molecular cloning and characterization of the human folate binding protein cDNA from placenta and malignant tissue culture (KB) cells. J Biol Chem, 1989, in press.

Elwood, PC, Knight, CB, Chabner, BA. Cloning of the cDNA for the human membrane-associated folate binding protein from placental and malignant nasopharyngeal carcinoma. Blood 1988;72:73.

Knight, BA, Chabner, BA, Elwood, PC. Studies of the phosphorylation of the membrane-associated folate binding protein (M-FBP) in human nasopharyngeal carcinoma (KB) cells. Blood 1988;72:274.

Knight, CB, Elwood, PC, Chabner, BA. Future directions for antifolate drug development. Adv Enz Reg, 1989, in press.

Elwood PC, Reid, WK, Marcell PD, Allen RH, Kolhouse J.F. Determination of the carbohydrate composition of mammalian glycoproteins by capillary gas chromatography/mass spectrometry. Anal Biochem 1988;175:202-11.

Kane MA, Elwood PC, Portillo RM, Anthony AC, Waxman S, Kolhouse JF. Influence on immunoreactive folate-binding proteins of extracellular folate concentration in cultured human cells. J. Clin. Invest 1988;81:1398-1406.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06719-01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Signal transduction events and the regulation of cell growth

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Jane B. Trepel

Principal Investigator

MB, COP, DCT, NCI

Wei-Gang Fang

Visiting Fellow

MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

NCI-Navy Oncology Branch, COP, DCT, NCI; Lombardi Cancer Center, Georgetown University

## LAB/BRANCH

Medicine Branch

## SECTION

Molecular Cell Biology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.5

## PROFESSIONAL:

1.5

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The signal transduction events regulation tumor cell growth are poorly understood. Recently, there has been an explosion in understanding of the signal transduction pathways associated with mitogenic events in model systems such as mouse 3T3 fibroblasts. Generally, this knowledge has not been utilized for studying human malignant disease. We have investigated the signal transduction events associated with the growth of small cell lung cancer (SCLC) in vitro. We found that two classes of SCLC cell lines could be differentiated: one class, subject to autocrine regulation by the mitogenic peptide gastrin releasing peptide (GRP), showed GRP-stimulated phosphatidylinositol turnover and a GRP-stimulated  $Ca^{2+}$  transient; the second class did not produce or respond to GSP. Oncogene analysis showed that the cells that produced and responded to GRP expressed the l-myc oncogene, while cells that were GRP-independent expressed c-myc or n-myc. These data suggested that an agent active in blocking GRP binding to its receptor would inhibit the growth of an SCLC cell line that produced and responded to GRP. Using a GRP-receptor antagonist with a novel reduced peptide bond structure, we showed inhibition of GRP-stimulated phosphatidylinositol hydrolysis, inhibition of GRP-stimulated  $Ca^{2+}$  flux, and inhibition of the growth of a GRP-responsive SCLC line. In addition, we demonstrated the interruption of growth-associated signal transduction pathways by active phorbol esters and by cholera toxin, and demonstrated the reversal of phorbol ester inhibition by depletion of intracellular protein kinase C. Currently, we are applying these techniques to the study of prostate cancer and hematopoietic neoplasms.



### Major Findings

1. The first demonstration of a signal transduction pathway in small cell lung cancer.
2. Inhibition of the *in vitro* growth of lung cancer cell line associated with blockade of a mitogenic signal transduction pathway.

### PUBLICATIONS

Trepel JB, Moyer J, Heikkila R, Sausville EA. Modulation of bombesin-induced phosphatidylinositol hydrolysis in a small cell lung cancer cell line. *Biochem* 1988;255:403-10.

Minna JD, Cuttitta F, Battey JF, Mulshine JL, Gazdar AF, Trepel J.B, Sausville EA. Gastrin-releasing peptide and other autocrine growth factors in lung cancer: pathogenic and treatment implications. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. *Important advances in oncology*. Philadelphia: J.B. Lippincott, 1988;55-64.

Sausville EA, Miller JD, Heikkila R, Neckers LM, Trepel JB. A correlation of bombesin-responsiveness with myc-family gene expression in small cell lung carcinoma cell lines. *Ann NY Acad Sci* 1988;547:310-21.

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Sausville EA, Trepel JB, Moyer JD. Inhibitors of bombesin-stimulated intracellular signals: interruption of an autocrine pathway as a therapeutic strategy. In: *International symposium on the biology and kinetics of surviving tumor*. New York: Alan R. Liss, 1989, in press.

Le-Bacq-Verhayden A-M, Trepel JB, Sausville EA, Battey JF. Bombesin and gastrin releasing peptide: neuropeptides, secretagogues, and growth factors. In: Sporn MB, Roberts AB, eds. *Handbook of experimental pharmacology Vol. 95/II--peptide growth factors and their receptors*. Heidelberg: Springer-Verlag, 1989, in press.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06720-01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT: *See instructions on page 1. This must fit on one line between the borders.*

Suramin and related substances in prostate cancer and glioblastoma

PRINCIPAL INVESTIGATOR: *List other professional personnel below the Principal Investigator. (Name, title, laboratory, and institute affiliation)*

Charles E. Myers, M.D.

Chief, Medicine Branch

COP,DCT,NCI

Renato V. LaRocca, M.D.

Principal Investigator

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Romano Danesi, M.D.

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## COOPERATING UNITS (if any)

## LAB/BRANCH

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Molecular Cell Biology Section

## INSTITUTE AND LOCATION

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## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

We have characterized the pattern of proto-oncogene and growth factor mRNA expression in a battery of human colorectal carcinoma and glioma cell lines. With regard to the glioma cell lines, analysis of mRNA expression of 6 proto-oncogenes revealed marked heterogeneity of expression between cell lines only for the *sis* proto-oncogene, which encodes for a protein having marked homology to the B chain of platelet-derived growth factor. The presence of detectable mRNA in these lines correlated in addition with the presence of a pleomorphic glial cell morphologic heterogeneity among glioblastoma cell lines may represent various points of arrest along a developmental pathway of the human astrocyte, with the presence of detectable levels of *sis* and GFAP mRNA correlating with a more differentiated phenotype.

We have tested a battery of these glioma lines with varying morphology with suramin. Our preliminary data shows that the presence of *sis* mRNA in a cell line serves as a marker for increased suramin sensitivity. In addition, the degree of *sis* mRNA signal intensity is also proportional to that cell line's LD50 to suramin. No significant variations in cell line morphology have been observed.

With regard to suramin sensitivity in human prostate and colorectal cell lines, there appears to be significant variation. In addition, with regard to prostate lines, suramin's growth inhibitory effect appears to be mediated via mechanisms which are not entirely androgen independent.

### Major Findings

- 1) There are variable levels of sis mRNA expression among glioma cell lines and the presence of detectable levels correlates with particular morphologic subtypes as well as often with detectable levels of GFAP mRNA. This suggests a possible developmental hypothesis in the origin of glioblastoma.
- 2) The presence of detectable sis mRNA in glioma cell lines serves as a marker for enhanced sensitivity of that line to suramin.
- 3) The sensitivity of colorectal and prostate cell lines to suramin appears variable and may be related to differences in expression of various growth factors.

### PUBLICATIONS

La Rocca RV, Rosenblum M, Westermarck B, Israel MA. Patterns of proto-oncogene expression in human glioma cell lines. J Neurosci Res, 1989, in press.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06721-01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of drug resistance by flow microfluorocytometry

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Jane B. Trepel	Principal Investigator	MB, COP, DCT, NCI
F. Wei-Gang	Visiting Fellow	MB, COP, DCT, NCI
L.M. Neckers	Research Chemist	MB, COP, DCT, NCI
K.H. Cowan, M.D., Ph.D.	Senior Investigator	MB, COP, DCT, NCI
P. Elwood, M.D.	Senior Investigator	MB, COP, DCT, NCI
A.T. Fojo, M.D.	Senior Investigator	MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

Pediatrics Branch, COP, DCT, NCI; Navy Medical Oncology Branch, NCI; Japanese Foundation for Cancer Research

## LAB/BRANCH

Medicine Branch

## SECTION

Molecular Cell Biology

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL:

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Since the discovery of the importance of the mdr-1 gene product in multidrug resistance, most of the studies of the multidrug resistance phenotype have been performed at the nucleic acid level. We have established assays to examine drug resistance at the protein level by using flow cytometry to look at intact single cells. There are significant advantages to this approach, especially in probing the role of P-glycoprotein expression in innate and acquired multidrug resistance in clinical specimens. The assays we have developed allow us to measure P-glycoprotein expression and adriamycin content in each of thousands of cells, within hours of receiving a specimen. We have developed a two-parameter technique that allows us to correlate expression and adriamycin content in individual cells in a tumor specimen or cell line. Using this technique, we have been able to identify a subpopulation of innately resistant cells (high P-glycoprotein expression, low Adr content) in a CML-blast crisis cell line that was not detectable by previously available single parameter techniques. Conversely, we were able to detect a drug-sensitive subpopulation in a CML-blast crisis cell lines selected for Adr resistance and in MCF-7 cells transfected with the mdr-1 gene.

In addition, we are working on flow cytometric analysis of other measures of drug resistance, including single cell detection of dihydrofolate reductase by fluoresceinated methotrexate and on the use of flow cytometry for the rapid screening of resistance reversal agents. We will use the techniques outlined to study drug resistance and its reversal in a variety of tumors. A project utilizing the unique multiparameter capabilities of flow analysis has been initiated to study the relationship of drug resistance to N-myc expression.

Major Findings:

Development of a highly sensitive dual parameter flow cytometric technique for detection of multidrug resistant cells within a tumor population.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06722-01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of IL6-mediated tumor growth

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Richard P. Nordan	Principal Investigator	MB, COP, DCT, NCI
G. Schwab	Visiting Fellow	MB, COP, DCT, NCI
F. D'Allesandro	Visiting Fellow	MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

S. Rudikoff, Laboratory of Genetics, NCI; L. Aarden, Red Cross Blood Transfusion Service, Amsterdam; J. Van Snick, Ludwig Institute, Brussels

## LAB/BRANCH

Medicine Branch

## SECTION

Tumor Cell Biology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

1.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The goal of this laboratory is to increase our understanding of how growth factor-dependent cells escape the control of regulatory growth factors thus becoming autonomous tumor cells. Previous studies by R. Nordan identified and characterized a new cytokine, interleukin 6 (IL6), that supported the growth of early murine plasma cell tumors. These early tumors also require an inflammatory peritoneal oil granuloma for in vivo growth. Without this microenvironment the cells fail to grow in vivo. The eventual progression of these tumors to a fully malignant phenotype in vivo is associated with a concomitant transition to IL6-independent growth in vitro. Our working hypothesis is that the early tumor cells require IL6 (supplied by the inflammatory microenvironment) for in vivo growth with the subsequent loss of IL6-dependence representing a key step in the progression to a fully malignant tumor. Since the establishment of this laboratory in the Tumor Biology Section in January, 1989, we have initiated studies aimed at elucidating the role of IL6 in human and murine plasma cell tumor growth and how these cells escape the requirement for this growth factor.

One approach we are taking is to determine if an autocrine loop is responsible for the transition to autonomous tumor cell growth and to analyze the events leading to this phenotype. We have identified human and murine myeloma cell lines that constitutively produce IL6. In addition, we have found that the in vitro growth of the human myeloma cell line, U266, can be partially inhibited with a neutralizing monoclonal antibody to human IL6 thus demonstrating the existence of an IL6-based autocrine loop. To determine if this loop is totally responsible for the autonomous growth of U266 we are investigating the use of antisense RNA as a means of down-regulating the endogenous production of IL6 and possibly reverting the cell to the growth factor-dependent phenotype. Thus far we have inserted the human IL6 gene into high level antisense expression vectors and expect to have stable transfectants of U266 in the near future. Projected studies will involve an attempt to identify the molecular event(s) responsible for the upregulation of endogenous IL6 production.

The progression to factor-independent growth may also involve a constitutive activation of some part of the signal transduction pathway which for IL6 is unknown. We have initiated the characterization of the starting point of this pathway: the IL6 receptor. Although we are in the early phases of this study we have identified three and possibly four distinct membrane proteins which interact with IL6 to form the putative receptor complex. Projected studies include the structural characterization of the receptor complex and the generation of monoclonal antibodies to components of the receptor leading to the molecular biological characterization of these molecules.

Another goal of this project has been the development of neutralizing monoclonal antibodies to murine IL6. These antibodies will be used in in vivo studies designed to test the hypothesis that IL6-dependent in vitro growth of early murine tumors extends to in vivo growth as well. Such antibodies will also be valuable in investigating the role of IL6 in IL1- and TNF-mediated responses in vivo. Thus far we have generated several monoclonal antibodies to murine IL6, however none of these inhibit the biologic activity of the molecule. Work in this area is continuing.

#### Major Findings:

1. We have identified an IL6-based autocrine growth mechanism in the human myeloma cell line, U266.
2. We have identified three possibly four distinct proteins which associate with IL6 on the cell surface and presumably constitute the IL6 receptor.

#### PUBLICATIONS

Neta R, Vogel S, Sipe JD, Wong GW, Nordan, RP. Comparison of in vivo effects of human recombinant IL-1 and human recombinant IL-6 in mice. *Lymphokine Res* 1988;7(4):403-12.

Mock BA, Nordan RP, Justice MJ, Kozak C, Jenkins NA, Copeland NG, Clark S, Wong G, Rudikoff S. The murine IL-6 gene maps to the proximal region of chromosome 5. *J Immunol* 1989;142:1372-6.

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Plaut M, Pierce J, Watson C, Hanley-Hyde J, Nordan, R, Takaki, S, Takatsu, K, Paul, W.E. Mouse mast cell lines produce interleukins in response to cross linkage of FcεRI or to calcium ionophores. *Nature* 1989;339:64-7.

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Van Snick J, Nordan .P. Interleukin 6. In: Habenicht A, ed. Growth factors differentiation factors and cytokines. Heidelberg: Springer-Verlag, 1989, in press.

Nordan RP, Mock BA, Neckers LM, Rudikoff, S. The role of plasmacytoma growth factor in the in vivo responses of murine plasmacytoma cells. *Ann NY Acad Sci* 1989;557:200-5.

Morrissey PJ, Goodwin RG, Nordan RP, Anderson D, Grabstein KH, Cossman D, Sims J, Lupton S, Acres B, Reed SG, Mochizuki D, Eisenman J, Conlon PJ, Namen AE. Recombinant IL7, pre B cell growth factor, has costimulatory activity on purified mature T-cells. *J Exp Med* 1989;169:707-16.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06723 01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Beta subunit of the interleukin-2 receptor in immature T cell neoplasms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

L.M. Neckers, Ph.D.	Principal Investigator	MB, COP, DCT, NCI
O.R. Colamonici, M.D.	Visiting Associate	MB, COP, DCT, NCI
J.B. Trepel	Biologist	MB, COP, DCT, NCI
D.G. Poplack	Senior Investigator	PB, COP, DCT, NCI
I. Kirsch, M.D.	Senior Investigator	NMOB, COP, DCT, NCI
A. Rosolen, M.D.	Visiting Fellow	MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

NCI-Navy Oncology Branch, COP, DCT, NCI; PB, COP, DCT, NCI

## LAB/BRANCH

Medicine Branch

## SECTION

Tumor Cell Biology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

4.0

## PROFESSIONAL

4.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)

We have studied 45 cases of T Cell and myeloid leukemia trying to further characterize them. In 15 cases, these cells respond to treatment with IL-2, in that they develop the ability to kill tumor cells. Their phenotype changes to resemble that of a more mature T cell. Using these cases, we have studied the role of the T cell receptor in generation of cytotoxic activity. In one case, IL-2 induced a proliferation of cells utilizing TCR  $\gamma\delta$ . We demonstrated that this TCR was functional in that anti-CD3 stimulated an elevation of intracellular  $[Ca^{2+}]$  and augmented MHC-unrestricted cytolysis. Triggering of the TCR  $\gamma\delta$  in this case leads directly to release of cytolytic granule enzymes.

In studying the role of IL-2 in this process, we observed that changes we recorded following IL-2 were transduced solely via the P75 IL-2 binding protein and not the P55 (TAC) protein. Based on these findings, we have begun a clinical protocol, in collaboration with Drs. Poplack and Kirsch, to study the effectiveness of rIL-2 in vivo as a treatment for immature T cell malignancies.

In studying LGL proliferations, normal thymocytes and T-ALL cells, we have found that IL-2 augments cytolytic activity solely through stimulation of the  $\beta$  subunit of the IL-2 receptor. An analysis of hematopoietic neoplasms by affinity cross-linking techniques reveal that immature cells of either T, B, or myeloid origin express only the  $\beta$  subunit of the IL-2 receptor, while more mature cells express both  $\alpha$  and  $\beta$  subunits. The  $\beta$  subunit is capable of signal transduction in immature T cells, LGL cells, BCLL, and B-WDL cells.

### Major Findings

- 1) We found T cell malignancies capable of IL-2 induced MHC-unrestricted killing.
- 2) The phenotype analyzed by using monoclonal antibodies showed similarity among all responding cases (T/myeloid stem cell).
- 3) In one well-studied case, IL-2 induced utilization of TCR  $\gamma\delta$  in induction of MHC-unrestricted cytotoxicity.
- 4) TCR  $\gamma\delta$  activation leads directly to release of cytolytic granule enzymes.
- 5) The responding cases do not possess the P55 (TAC) IL-2 binding protein but do express the P75 IL-2 binding protein.
- 6) IL-2 is capable of inducing phenotypic and functional maturation of immature T cell neoplasms via the P75 IL-2 protein.
- 7) We have established a clinical protocol to study the effectiveness of rIL-2 in vivo as a treatment for some immature T cell malignancies.
- 8) IL-2 stimulates MHC-unrestricted killing by normal and leukemia LGL cells via stimulation of the  $\beta$  subunit of the IL-2 receptor.
- 9) The  $\beta$  subunit of the IL2R, unlike the  $\alpha$  subunit, is widely expressed throughout the hematopoietic lineage.

### PUBLICATIONS

Colamonici OR, Quinones R, Rosolen A, Trepel JB, Sausville E, Phares JC, Gress R, Poplack D, Weber J, Schechter GP, Neckers LM. The beta subunit of the interleukin-2 receptor mediates the interleukin-2 induction of anti-CD-3-redirectioned cytotoxic capability in large granular lymphocytes. *Blood* 1988;71: 825-8.

Colamonici OR, Ang S, Quinones R, Henkart P, Heikkila R, Gress R, Felix C, Kirsch I, Longo D, Marti G, Seidman JG, Neckers LM. IL-2 dependent expansion of CD3<sup>+</sup> large lymphocytes expressing T cell receptor- $\gamma\delta$ . Evidence for a functional receptor by anti-CD3 activation of cytotoxicity. *J Immunol* 1988;140:2527-3.

Colamonici OR, Cole D, Rosolen A, Kirsch I, Felix C, Poplack DG, Jaffe ES, Neckers LM. Stimulation of the beta subunit of the IL-2 receptor induces MHC-unrestricted cytotoxicity in T acute lymphoblastic leukemia cells and normal thymocytes. *J Immunol* 1988;141:1202-5.

Rosolen A, Nakanishi M, Poplack DG, Cole D, Quinones R, Reaman G, Trepel JB, Cotelingam JD, Sausville EA, Marti GE, Jaffe ES, Neckers LM, Colamonici OR. Expression of interleukin-2 receptor beta subunit in hematopoietic malignancies. *Blood* 1989;73:1968-72.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06724 01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Cellular Oligonucleotide Uptakes

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

L. M. Neckers, Ph.D.	Principal Investigator	MB, COP, DCT, NCI
C. A. Stein, M.D.	Senior Investigator	MB, COP, DCT, NCI
J. S. Cohen, M.D.	Senior Investigator	MB, COP, DCT, NCI
L. Whitesell	Biotechnology Fellow	PB, COP, DCT, NCI

## COOPERATING UNITS (if any)

Pediatric Branch, COP, DCT, NCI

## LAB/BRANCH

Medicine Branch

## SECTION

Tumor Cell Biology Section

## INSTITUTE AND LOCATION

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## TOTAL MAN-YEARS

2.0

## PROFESSIONAL

1.0

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to be able to rationally design oligonucleotides which are stable in culture and penetrate cells more efficiently than normal oligos, we have investigated the mechanism by which cells transport normal oligomers. Using acridine-labeled oligos and flow cytometry, we found that transport is active, receptor-mediated and energy dependent. We elucidated the characteristics of an oligonucleotide which are critical for uptake and studied the ability of certain oligonucleotide derivatives to compete for this uptake process. We found that methylphosphonates do not enter cells via this mechanism, but that phosphorothioates do, although much less efficiently than normal oligos. Uptake is by endocytosis and generally results in the occurrence of oligo-containing vesicles in the cytoplasm. In general, oligos are localized to cytoplasm and not the nucleus following uptake. By fluoresceinating novel oligo derivatives, one can easily follow their rate of accumulation, or lack thereof, by cells. In this way, more efficient oligos can be rapidly designed and tested.

Using liposomes of various types, we have explored the targeted delivery of oligos to cells. The use of antibody-coated pH-sensitive liposomes is especially promising as a method of delivering oligos to the cytoplasm, and not lysosomes, of specific cells. Other methods of delivery, such as polymer encapsulation, are currently under investigation.

Major Findings:

1. We are the first to describe the nature of the cellular uptake system for oligomers.
2. We have identified an 80 kD membrane protein which may mediate this process.
3. We have shown that, using liposomal fusion, targeted delivery of both the normal and substituted oligos to cells is possible.
4. We have demonstrated the potential utility of phosphorothioate oligos as anti-oncogene compounds.

PUBLICATIONS

Loke SL, Stein CA, Zhang XH, Mori K, Nakanishi M, Subasinghe C, Cohen, JS, Neckers, LM. Characterization of oligonucleotide transport into living cells. *Proc Natl Acad Sci USA* 1989;86:3474-8.

Loke SL, Stein CA, Zhang, XH, Avigan M, Cohen JS, Neckers LM. Delivery of c-myc antisense phosphorothioate oligodeoxynucleotides to hematopoietic cells in culture by lysosome fusion: specific reduction in c-myc protein expression correlates with inhibition of cell growth and DNA synthesis. *Curr Top Immunol Microbiol* 1988;141:282-9.

Stein CA, Mori K, Loke SL, Subasinghe C, Shinozuka K, Cohen JS, Neckers LM. Phosphorothioate and normal deoxynucleotides with 5'-linked acridine: characterization and preliminary kinetics of cellular uptake. *Gene* 1988;72:333-41.

Neckers M. Antisense oligonucleotides: mechanism of cellular uptake and utility as inhibitors of oncogene expression. In: Cohen JS, ed. *Antisense inhibitors of gene expression*. London: Macmillan Press, in press, 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 CM 06725 01 M
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Inhibition of N-myc expression in neuroblastoma cell lines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
L.M. Neckers, Ph.D. A. Rosolem, M.D. L. Whitesell	Principal Investigator Visiting Fellow Biotechnology Fellow	MB, COP, DCT, NCI MB, COP, DCT, NCI MB, COP, DCT, NCI
COOPERATING UNITS (if any)  Pediatric Branch, COP, DCT, NCI		
LAB/BRANCH Medicine Branch		
SECTION Tumor Cell Biology Section		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 2.5	PROFESSIONAL 2.5	OTHER 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>             N-myc is a nuclear oncogene which is generally overexpressed in neuroblastoma. Although N-myc protein is DNA binding and is structurally similar to c-myc, its function in both normal and neoplastic cells is unknown. We are making use of the "antisense concept" to study the role of N-myc in neuroblastoma cell lines which overexpress this oncogene. The approach is two-fold: 1) addition to cell cultures of small single-stranded oligonucleotides complementary to a portion of the N-myc gene in antisense orientation. Using the former approach, we have been able to show that oligonucleotide 15-mers complementary to an area containing the initiation site of N-myc RNA are capable of concentrating in cells and inhibiting production of N-myc protein. In addition, these oligos inhibit cellular DNA synthesis and production of a nuclear protein termed Ki67. This protein may be a co-factor for DNA polymerase alpha and is required for DNA synthesis in isolated nuclei. Continued administration of antisense oligomer to neuroblastoma cells leads to reduced cell growth. We are currently studying the state of differentiation of these cells following antisense administration.           </p>		

In order to utilize the second approach, vector transfection, we have constructed an antisense *N-myc* containing episomal vector which replicates extra-chromosomally at high copy number. In a similar vein, we have perfected a method to insert a small oligonucleotide of chosen sequence into this vector. This methodology will be used to construct nuclear replicating ribozyme sequences specific for *N-myc* and to use such a vector to interfere with intranuclear processing of *N-myc* transcripts.

#### Major Findings

1. Addition of *N-myc* antisense oligomers to neuroblastoma cells in culture reduce the amount of *N-myc* protein which is detectable.
2. DNA synthesis is also reduced by this treatment and cell growth is slowed.
3. The nuclear protein, Ki67 is also reduced by *N-myc* antisense treatment. This protein is required for DNA synthesis in isolated nuclei.
4. *N-myc* protein may regulate Ki67 gene expression in neuroblastoma cells.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06726 01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of the role of RNase in vivo in modulation of antisense action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

L.M. Neckers, Ph.D.

Principal Investigator

MB, COP, DCT, NCI

A. Rosolen, M.D.

Visiting Fellow

MB, COP, DCT, NCI

E. Kyle

Microbiologist

MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Medicine Branch

## SECTION

Tumor Cell Biology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.5

## PROFESSIONAL

1.5

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The intracellular mechanism of action of exogeneously administered antisense oligonucleotides is not known. Two mechanisms have been suggested based on studies in cell free systems. These are: 1) inhibition of ribosome attachment or movement along mRNA; and, 2) creating a substrate for RNase H degradation of targeted mRNA. RNase H is an enzyme which destroys the RNA portion of an RNA/DNA hybrid complex and is present in the cytoplasm of all proliferating cells. Its natural function in cells is unknown. We have obtained a full-length cDNA coding for bacterial RNase H. We will place this cDNA into a mammalian expression vector which replicates episomally and thus attains high copy number. Cells transfected with this construct and sham-transfected cells will be compared for their responsiveness to antisense oligonucleotide addition. If RNase H is involved in the intracellular functioning of antisense oligos, then cells with high levels of RNase H should respond better to addition of these compounds. Likewise, the RNase H cDNA in reverse orientation in the vector will be transfected in a similar experiment. In this case, endogenous RNase production should be inhibited (due to this antisense vector) and response to other antisense oligos added extracellularly should be reduced.

Major Findings

1. RNAse H potentiates the efficacy of antisense oligos in a cell free system.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06727 01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

Oncogene activation in human malignancies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name title, laboratory, and institute affiliation)

Maria Zajac-Kaye, Ph.D.	Principal Investigator	MB, DCT, NCI
B.W. Yu, M.D.	Clinical Associate	MB, DCT, NCI
N. Ben-Baruch, M.D.	Clinical Associate	MB, DCT, NCI

## COOPERATING UNITS (if any)

D. Levens, M.D., Laboratory of Pathology, DCBD, NCI, NIH

## LAB/BRANCH

Medicine Branch

## SECTION

Tumor Cell Biology

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.0

## PROFESSIONAL

2.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The investigations of therapies for opportunistic infections is focused on the interactions of antifolate agents on the metabolic pathways in toxoplasma gondii and pneumocystis carinii. In addition to the use of basic biochemical technologies, we are using the tools of molecular biology to provide quantities of the critical enzymes for characterization and to aid in the search for new therapeutic agents.

We have investigated the mechanisms of oncogenes regulation in human malignancies. Deregulation of the c-myc gene accompanies all cases of human Burkitt's lymphoma (BL) and therefore the mechanism underlying the transcriptional regulation of the c-myc gene in lymphoid tumors is of great importance. We have recently discovered a cis element located in the intron I of the human c-myc gene which binds a novel nuclear protein and we showed that this binding was abolished by point mutation present in the corresponding region in most BL DNA. We have now purified and identified this Myc Intron Factor (designated MIF) to be a 138 kD protein. We have also demonstrated that the 138kD MIF is a phosphoprotein and that phosphorylation of MIF is required for the interaction with its recognition sequence. Our results suggest that the intron I cis element, the binding factor MIF and the kinases which phosphorylates MIF may comprise an important physiological circuit, alteration of which may perturb c-myc expression and may have malignant consequences.

In addition we have studied the effect of cancer agents on the regulation of transcription factors. We have shown that treatment of cells with certain pharmacologic agents alters dramatically MIF binding pattern to the intron I *cis* element. Understanding the process by which transcription factors regulate gene expression will allow development of reagents which could turn off uncontrolled expression of genes implicated in malignant transformation.

### Major Findings

The mechanism of transcriptional deregulation of oncogenes in human malignancies was investigated. The major projects were the following:

1. Mechanism involved in the transcriptional regulation of *c-myc* oncogene in lymphoid tumors. The *c-myc* oncogene is deregulated in many types of human tumors and therefore is an excellent model system for studying the effects of transcriptional deregulation in human tumorigenesis. In studying *c-myc* deregulation in a Burkitt's lymphoma (BL) cell line derived at the NIH from an AIDS patient, a 20 bp region located in the first intron of the normal *c-myc* gene was identified as a binding site for a novel nuclear protein. We showed that this binding was abolished by a point mutation in a corresponding region of the *c-myc* gene derived from the BL DNA. Moreover, this region is mutated in the majority of BL.

To understand the role of the intron I sequence in the regulation of the *c-myc* gene purification and identification of this protein was undertaken. A 138 kD nuclear protein was identified as the factor which binds to the previously identified 20bp *cis* element located in the intron I of the *c-myc* gene. This Myc Intron Factor (termed MIF) binds to the wild type *c-myc* sequence but does not bind to the *c-myc* from Burkitt's lymphoma which contain point mutations in this binding region. We have also demonstrated that the 138 kD MIF is a phosphoprotein and that treatment of the purified MIF with potato acid phosphatase abolished binding to its 20bp *c-myc* recognition sequence. Binding activity was protected by inclusion of phosphatase inhibitors. These results suggest that phosphorylation is required for the specific DNA:MIF interaction *in vitro* and that the phosphorylation state of MIF may be an important factor in controlling *c-myc* expression *in vivo*. Production of antibody to MIF as well as cloning of the gene which encodes this protein will allow us to better understand its role in the regulation of the *c-myc* gene and in oncogenesis.

2. Effect of cancer agents on the function of transcription factors which regulate expression of cellular oncogenes. HL60 cells provide an experimental system where *c-myc* expression (linked with cell growth and differentiation) can be physiologically modulated and are easily examined. In response to agents which differentiate HL60 cells the *c-myc* expression dramatically decreases. We have observed dramatic and reproducible differences in DNA protein complexes of the *c-myc* in response to treatment with retinoic acid and suramin. Studies are under way to determine whether suramin or retinoic acid alters the form of these nuclear proteins and thus activating them to allow specific binding to DNA (for example: by changing phosphorylation state). In particular, experiments to determine whether suramin downregulates *c-myc* transcription by either a transcriptional or post transcriptional mechanism is in progress (done in collaboration with Len Neckers). Similar experiments will be conducted using prostate cell lines which overexpress the *c-myc* oncogene.

PUBLICATIONS

Zajac-Kaye M, Gelmann E, Levens D. A point mutation in the *c-myc* locus of a Burkitts lymphoma abolishes binding of a nuclear protein. Science 1988;240:1776-89.

Zajac-Kaye M, Avigan M, Takimoto M, Pitaluga S, Quinn J, Gelmann E, Levens D. Multifactorial regulation of the human *c-myc* oncogene. In: Potter M, Melchers F, eds. Current topics in microbiology and immunology; mechanism in B cell neoplasia. Berlin: Springer-Verlag, 1988;247-52.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06728 01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Regulation of Tyrosine Kinases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ivan D. Horak, Ph.D.	Principal Investigator	MB, DCT, NCI
F. Gregory	Microbiologist	LTVB, DCE, NCI
E.M. Horak	Microbiologist	LTVB, DCE, NCI
L.M. Wahl, M.D.	Senior Investigator	NIDR
R.E. Gress, M.D.	Senior Investigator	EIB, DCBD, NCI
M. Popovic, M.D., Ph.D.	Senior Investigator	LTCB, DCE, NCI

## COOPERATING UNITS (if any)

Laboratory of Tumor Virus Biology, DCE, NCI

## LAB/BRANCH

Medicine Branch

## SECTION

Molecular Cell Biology

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

2.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Proto-oncogenes encode protein that comprise a select group of cellular regulatory proteins, whose mutation or aberrant expression can result in oncogenic transformation. More than half of all known proto-oncogenes encode tyrosine-specific protein kinases. Protein-tyrosine kinases of src family may be viewed as a component of a signal transduction cascade on which activation of a cell surface activation of a cell surface receptor stimulates phosphorylation of a set of important regulatory protein in a cell. The aim of the research within this project is to define the normal function and regulation of the src family of tyrosine kinases in normal and neoplastic hematopoietic cells. Towards this goal, as we have taken steps to prepare molecular and immunologic reagents and set up elutriation as a resource of normal hematopoietic cells to analyze the expression and activity of src family genes and their protein product in normal fresh human cells and leukemia/lymphoma cell lines. Using murine and human T-lympocytes, Dr. Bolen's laboratory discovered that LCK gene product is physically associated with both CD4 and CD8 surface glycoproteins. These receptors are important for T-cell signal transduction. Most notably, the CD4 surface protein also acts as the cellular receptor for the human immunodeficiency virus (HIV).

Objectives: Analysis of the src family tyrosine protein kinase members in normal and transformed cells.

Mechanism of regulation of the src family of tyrosine protein kinase members in normal and transformed cells.

Methods Employed:

Cell culture

Propagation of normal clonotypic T lymphocytes

Fluorescence-activated cell surface analysis

Differential isolation of normal peripheral hematopoietic cells by elutriation

Generation of peptide-specific polyclonal rabbit antisera

Immunoprecipitation and protein kinase assays

Immunoblot analysis

Phosphoamino acid analysis

One and two-dimensional gel electrophoresis

Major Findings:

We utilized cellular systems in the study of the regulation and function of the src family of tyrosine protein kinases in human and murine lymphocytes.

Using T lymphocytes, we have discovered that one member of the src family, p56<sup>lck</sup> is physically associated with both the CD4 and CD8 T-cell surface glycoproteins and can be modified by serine kinases in response to T cell activation signals.

PUBLICATIONS

Veillette A, Horak ID, and Bolen JB. Post-transcriptional alterations of the tyrosine kinases p56<sup>lck</sup> in response to activators of protein kinase C. *Oncogene Res* 1988;2: 385-401.

Veillette A, Horak ID, Bolen JB. Alterations of the lymphocyte-specific protein kinase p56<sup>lck</sup> during activation. *Mol Cell Biol* 1988;8:4353-61.

Veillette A, O'Shaunessy J, Horak ID, Israel MA, Yee D, Rosen N., Fujita DJ, Kung, H., Biedler J.L, Bolen, JB. Coordinate alteration of the pp60<sup>c-src</sup> abundance and c-src RNA expression in human neuroblastoma variants. *Oncogene* 1989;4:421-9.

Horak ID, Kawakami T, Gregory F, Robbins KC, Bolen JB. Association of p60<sup>lyn</sup> with middle tumor antigen in murine polyoma transformed rat cells. *J Virol* 1989;63: 2343-7.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06729 01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth factor characterization of adrenal cancer cell lines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Charles E. Myers, M.D.	Chief, Medicine Branch	COP, DCT, NCI
Renato V. LaRocca, M.D.	Principal Investigator	MB, COP, DCT, NCI
Romano Danesi, M.D.	Guest Researcher	MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

NCI-Navy Oncology Branch, COP, DCT, NCI

## LAB/BRANCH

Medicine Branch

## SECTION

Molecular Cell Biology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have established and characterized the first human adrenocortical cell line (H-295) that expresses multiple pathways of steroid biosynthesis and is capable of growth in defined media. Growth, cytogenetic and ultrastructural features of this line have been defined. The steroids secreted by this line include pregnenolone, 17-hydroxypregnenolone, DHA, aldosterone and 11-deoxycortisol.

In addition, we have characterized the expression of a variety of proto-oncogenes and growth factors by this cell line as well as in the poorly differentiated adrenocortical cell line SW-13 and a battery of adrenal cancer tumor specimens. Of note is the presence of elevated levels of insulinlike growth factor 2 (IGF-2) mRNA found in this line and absence of detectable basic FGF transcripts. The presence of elevated levels of IGF-2 message were detected in the four adrenocortical carcinoma tumor specimens.

In light of our ongoing clinical protocol evaluating the efficacy of suramin in patients with a variety of malignancies including adrenal cancer, these two adrenocortical carcinoma cell lines have been tested for their sensitivity to this agent. By clonogenic assay, the LD50 for suramin appears to be above 400 micrograms/ml in both lines, which implies minimal antitumor effect at clinically achievable levels (up to 300 micrograms/ml). However, in the case of H-295, steroid biosynthesis can be effectively blocked at suramin concentrations as low as 100 micrograms/ml.

Major Findings

- 1) H-295 is an adrenocortical cell line capable of growing in defined media and secreting a variety of steroid hormones.
- 2) Elevated levels of IGF-2 mRNA are found in adrenocortical cancer.
- 3) Adrenal cell lines appear relatively insensitive to suramin.
- 4) Suramin is capable of blocking steroid hormone production by this cell line at relatively low concentrations.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06730 01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Polyanions used as anti-neoplastic and anti-HIV agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Charles E. Myers, M.D.	Chief, Medicine Branch	COP, DCT, NCI
Cy A. Stein, M.D.	Principal Investigator	MB, COP, DCT, NCI
Peter Brett, M.D.	Clinical Associate	MB, COP, DCT, NCI
Daniel Geselowitz, Ph.D.	Biotechnology Fellow	MB, COP, DCT, NCI
Zhen Hong-Li	Visiting Fellow	MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

## LAB/BRANCH

Medicine Branch

## SECTION

Molecular Cell Biology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The use of polyanions as anti-neoplastic and anti-HIV agents was investigated. Examples of these types of compounds are phosphorothioate oligodeoxynucleotides and the bis-naphthalene sulfonic acids (e.g. suramin). Specifically, the mechanism involved in the HIV-1 cytoprotective effect of phosphorothioate oligodeoxynucleotides and the effects of suramin on cell growth and regulation were investigated.



Major Findings

1. Mechanism involved in the HIV-1 cytoprotective effect of phosphorothioate oligodeoxynucleotides.

Molecules of this type can block the cytopathic effect of HIV-1 in de novo-infected ATH 8 cells. We have demonstrated that this non-sequence specific effect is a general one and that virtually any phosphorothioate oligomer is active at a concentration of 1-10  $\mu$ M. We then investigated the ability of phosphorothioates to bind to CD4. It was shown by means of a modified gel retardation assay that S-dC28 and S-dC15 but not S-dC5 could bind to the CD4. Furthermore, binding at the S-dC28 to CD4 blocked the binding of the HIV-1 envelope protein gp 120. The binding of MoAbs that recognized the HIV-1 binding site were also affected. Because of this property, phosphorothioate oligomers were shown to be potent inhibitors of syncytia formation in the CEM 50/MOLT 3/IIIB system. We are currently in the process of phosphorodithioate analogs to see whether the maximal inhibitory effect can be achieved at shorter chain length.

2. Effects of suramin on cell growth and regulation.

We have shown that suramin is an active agent in a variety of solid tumors including lymphomas and hormonally unresponsive prostate cancer. We have studied the effects of suramin in HL 60 cells and have found that suramin profoundly affects growth and causes partial cellular differentiation. Levels of transferrin receptor and transferrin receptor mRNA were also decreased by suramin and the effects of 1 mM cAMP on HL 60 differentiation was also blocked. Future studies are planned to synthesize analogs of suramin. Some of these may have greater specificities for individual growth factors and/or different mechanism of cellular uptake. The combination of suramin and other anti-neoplastic agents will be studied in a variety of tumor cell lines in order to guide the eventual implementation of clinical trials.

PUBLICATIONS

Stein CA, LaRocca R, McAtee N, Thomas, Horne M, Myers CE. Suramin--an anti-cancer drug with a unique mechanism of action. *J Clin Oncol* 1989;7:499-508.

Stein CA, Mori K, Loke SL, Subasinghe C, Shinozuka K, Cohen JS, Neckers LM. Phosphorothioate and normal oligodeoxynucleotides with 5'-linked acridine: characterization and preliminary kinetics of cellular uptake. *Gene* 1989;72:333-41.

Loke SL, Stein CA, Zhang X, Mori K, Nakanishi M, Subasinghe C, Cohen JS, Neckers LM. Characterization of oligonucleotide transport into living cells: identification of an 80 kD binding protein by oligo dT-cellulose affinity chromatography. *Proc Natl Acad Sci* 1989;86:374

Majumdar C, Stein CA, Cohen JS, Boder S, Wilson, S. HIV reverse transcriptase step-wise mechanism: phosphorothioate oligodeoxynucleotide as primer. *Biochemistry* 1989;28:1340-6.

Gao W, Stein, CA, Cohen JS, Dutschman G, Cheng Y-C. Effect of phosphorothioate oligodeoxynucleotides on herpes simplex virus type 2 induced DNA polymerase. *Biochemistry* 1989, in press.

Cazenave L, Stein CA, Loreau N, Thuong N, Neckers L, Subasinghe C, Helene C, Cohen JS, Toulme, J-J. Comparative inhibition of rabbit globin mRNA by modified antimesenger oligodeoxynucleotides. *Nucl. Acids Res.*, in press, 1989.

Stein CA, Matsukua M, Subasinghe C, Cohen JS, Broder S. Phosphorothioate oligodeoxynucleotides are potent non-sequence specific inhibitors of the HIV cytopathic effect in de novo infected cells. *AIDS Res. and Human Retroviruses* 1989, in press.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06731-01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Expression and regulation of the mdrl gene and transforming growth factor alpha

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Susan Bates, M.D.	Principal Investigator	MB, COP, DCT, NCI
Liz Deutsch	Biologist	MB, CIP, DCT, NCI
Yi-Nan Chen	Visiting Fellow	MB, COP, DCT, NCI
Gi-Ming Lai	Visiting Associate	MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

## LAB/BRANCH

Medicine Branch

## SECTION

Experimental Therapeutics Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Previous studies in Dr. Tito Fojo's laboratory demonstrated that mdr-1/P-glycoprotein could be regulated by differentiating agents in colon carcinoma. We have undertaken to extend these results by examining the same question in human neuroblastoma before and after treatment with differentiating agents. We have observed the induction of P-glycoprotein and mdr-1 RNA after retinoic acid treatment and have observed that this induction again is not accomplished by decreased drug accumulation, as expected. This result also observed by Dr. Fojo in two of four human colon carcinoma cell lines after treatment with sodium butyrate.

Currently, our studies in this area follow two diverging paths:

1. Correlation of mdr-1 expression with differentiation in human neuroblastoma tumor samples, rather than cell lines.
2. Studies aimed at understanding the failure of the induced P-glycoprotein to perform drug efflux.

These studies will include examination of the state of phosphorylation of P-glycoprotein before and after differentiating agent treatment; examination of "native" P-glycoprotein from normal colonic tissue for function and state of phosphorylation; and potential biochemical modulation of the state of phosphorylation by other agents followed by determination of drug accumulation.

### Studies of expression and regulation of the *mdr-1* gene (75% of time)

I. Studies of the *mdr-1* gene expression in human breast cancer. Efforts to identify expression of the *mdr-1* gene in untreated breast cancer led to the use of the technique of polymerase chain reaction to demonstrate the presence of low levels of *mdr-1* expression in all samples. RNA *in situ* hybridization subsequently localized this expression to stromal elements including myoepithelial cells, rather than to the malignant cells. Utilizing clinical samples obtained from the Medicine Branch protocol MB-228, expression of the *mdr-1* gene has been determined to be at low levels in the NCI's advanced, heavily pretreated population of patients. While this represents the NCI group of "multidrug resistant patients," these patients have been typically treated with a spectrum of agents not known to be involved in induction of the classical P-glycoprotein-mediated resistance phenotype. Future directions for this clinical protocol and for this research will be aimed at narrowing the scope of the study to include patients with a higher likelihood of having P-glycoprotein expression. Evaluation of clinical samples continues to be a major endpoint of this study. To this end, collaborations have been established to perform flow cytometric analysis of dispersed tumor samples before and after treatment with amiodarone, the P-glycoprotein blocking agent used in the protocol.

II. Studies of intrinsic and acquired drug resistance in human colorectal carcinoma. Selection of three different human colon carcinoma cell lines in adriamycin allowed us to examine intrinsic and acquired drug resistance in the lines. The first of the lines to be selected, SW 620, demonstrated very low levels of *mdr-1* in the parent, and exquisite adriamycin sensitivity. Selection in adriamycin resulted in overexpression of P-glycoprotein and *mdr-1* and concomitant decreased drug accumulation. Verapamil blocks drug efflux with a dose-response curve which varies according to the level of P-glycoprotein in the cell. The DLD-1 cell line also developed P-glycoprotein upon selection with adriamycin. However, the parental cell line was 78-fold more resistant than the parental SW 620 line. This resistance was not reversible with Verapamil, was related to mechanisms other than overexpression of the *mdr-1* gene, and was reversed by treatment with the glutathione reducing agent, BSO. The third cell line has failed to develop significant P-glycoprotein-mediated resistance despite reaching a level of adriamycin which in the SW 620 cell line resulted in more than 100-fold overexpression of the *mdr-1* gene. This model system has major implications for the ongoing Medicine Branch studies in colon carcinoma. It suggests that despite the prevalence of P-glycoprotein expression in primary human colon carcinoma, the drug resistance seen in untreated patients is most likely to be multifactorial. Future directions with this model system will be directed toward: 1) identifying other mechanisms of resistance; 2) determination of the effects of differentiating agents on the adriamycin resistant cell lines; and 3) identification of more potent reversing agents given the multifactorial nature of the resistance observed in these cell lines.

### Studies of expression and regulation of transforming growth factor $\alpha$ (25% of time)

I. Recent studies in the laboratory have provided direct evidence for the existence of an autocrine loop for the TGF $\alpha$  and the EGF receptor in proliferating normal breast epithelial cells. This is the first demonstration of such a loop in normal breast cells, and is the first in any normal system to show that blockade of the EGF receptor by antibodies results in decreased expression of TGF $\alpha$  and decreased growth. These studies have implications for the treatment of malignancy with EGF receptor antibodies, or other agents

which interfere with ligand/receptor interaction: if the autocrine loop is necessary for the maintenance of normal levels of cell division in certain cell systems in the body, then such therapy could be expected to interfere with those normal cell systems. Second, the studies imply that the notion that autocrine loops may be a means by which cancer cells escape from normal growth regulation may be partially in error. Malignant cells may appropriate normal autocrine loops, may alter them in some way to provide deranged signal transduction, or may escape from them altogether. Future directions for these studies: 1) determination of whether EGF receptor expression is autoregulated as TGF $\alpha$  level is, by activity of the autocrine loop; 2) determination of activity of the autocrine loop in vivo. We have already analyzed normal and benign disease breast samples by RNA in situ hybridization for TGF $\alpha$ . The studies should be extended to examine levels of TGF  $\alpha$  protein, and EGF receptor.

II. Examination of the TGF $\alpha$ /EGF receptor in a second cell system, colon cancer, has yielded results which support the observations made above. Differentiation of human colon cancer cell lines with sodium butyrate results in enhanced expression of TGF  $\alpha$  and EGF receptor. While differentiation accomplished by sodium butyrate is so accompanied by growth inhibition that EGF effects are not discernible, the lines which are inherently well-differentiated are the ones which respond to EGF with increased growth. While these studies are far from demonstrating the presence of an autocrine loop, they are consistent with the presence of a n autocrine TGF  $\alpha$ /EGF receptor loop in well-differentiated colon cancer. Future directions for these studies include: 1) examination of a normal colon cell line for expression of TGF $\alpha$  and EGF receptor (normal colon tissue expresses high levels of the latter); 2) examination of the effects of EGF receptor blocking agents in the well-differentiated colon cancer lines. If the autocrine loop exists in normal tissue, then it may be operant in well-differentiated malignancy as well.

III. Selection of an adriamycin-resistant MCF-7 line in Dr. Tito Fojo's laboratory resulted in the induction of both TGF  $\alpha$  and EGF receptor. Both the mechanism behind this induction, and its role in the resistant phenotype are subjects for this study. Growth factors could play a potential role in drug resistance by either protecting the cell from acute injury, or by aiding cell populations in recovery from injury. Adriamycin has been shown to decrease EGF receptor binding. If this resulted in decreased mitogenic stimulation by EGF, then the cells could potentially have prolongation of time in G<sub>0</sub> during chemotherapy, thus increasing the cells' refractoriness to treatment. Alternatively, growth factors could stimulate cell growth and recovery after chemotherapy, and so enhancing relative drug resistance. Directions for this study: 1) determination of sensitivity of the drug resistant cells to EGF/TGF $\alpha$  and to EGF receptor blocking agents as well; 2) determination either mixing a population a drug resistant cells with drug sensitive cells decrease the cell kill; and 3) evaluation of the mechanism of induction of TGF $\alpha$  and EGF receptor. Is adriamycin directly responsible on an ongoing basis? Revertants clearly have lower levels of both growth factor and receptor. Does this reduction occur acutely or coincidentally with loss of drug resistance?

PUBLICATIONS

Bates SE, Mickley LA, Richert N, Rudick J, Fojo AT. Expression of a drug resistance gene in human neuroblastoma cell lines: modulation by retinoic acid-induced differentiation. *Mol Cell Biol*, in press.

Mickley LA, Bates SE, Richert, ND, Rosen N, Fojo AT. Modulation of the expression of a multi-drug resistance gene (mdr/P170) by differentiating agents. *J Biol Chem*, in press.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06732 01 M

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Modulation of the expression of a multidrug resistance gene (mdr-1)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Antonio Fojo, Ph.D.

Principal Investigator

MB, COP, DCT, NCI

Lyn A. Mickley

Biologist

MB, COP, DCT, NCI

Yi-Nan Chen, M.D.

Visiting Fellow

MB, COP, DCT, NCI

## COOPERATING UNITS (if any)

## LAB/BRANCH

Medicine Branch

## SECTION

Experimental Therapeutics Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unraduated type. Do not exceed the space provided.)

Major Laboratory Focus: Drug Resistance

Summary of ongoing effort

Multidrug resistance mediated by the MDR-1 gene which encodes P-glycoprotein

This has been and continues to be a major focus of our laboratory efforts. Our work in the past year has focused on trying to further understand the factors that modulate the function of P-glycoprotein and has also included extensive work directed at establishing the role of the protein in clinical drug resistance.

## MAJOR FINDINGS

1. We have been involved in the the conduct of clinical trials to evaluate the role of P-glycoprotein antagonists in modulating clinical drug resistance in patients with colon cancer, renal cell carcinoma, and cancers of the adrenal gland. The protocol has been successfully conducted with accrual of nearly 50 patients in the past year. Preliminary results indicate that additional work will need to be done before significant advances can be made in these diseases. The key to the success of this and future protocols will be the ability to successfully measure *mdr-1*/Pgp in small clinical samples. In order to do this, we successfully developed in our laboratory, the technique of in-situ hybridization with RNA probes as the most sensitive technique available for measuring expression of *mdr-1* in small clinical samples. Entry one of these protocols required the availability of tissue and in most patients this was obtained by percutaneous needle aspirations or biopsies and expression of *mdr-1* was successfully measured in all samples so obtained.
2. Additional studies with clinical applications have been pursued aggressively and have focused on the identification of agents capable of reversing drug resistance. We have identified mitotane as an agent with potential clinical utility and have shown its mechanism of action to include the ability to increase drug accumulation independent in part of Pgp antagonism. This offers the possibility for synergism with other agents known to be inhibitors of Pgp function. In addition, our efforts are now focusing on identifying synergistic combinations that do not have additive toxicity with the goal of using these in clinical trials in patients with relapsed lymphoma.
3. Evidence generated in our laboratory suggesting that Pgp may not function in drug efflux in all cases and a report in the literature that different Pgps can modulate different phenotypes has the formed the basis for ongoing studies examining the primary structures of P-glycoprotein from normal tissues and clinical samples as well as cell lines selected for drug resistance and cell lines with intrinsic elevations of Pgp. To date, these studies have failed to demonstrate that other explanations must be sought to explain these observations.
4. Analysis of tumor specimens by in-situ hybridization with RNA probes has been developed in the laboratory and has been used successfully in clinical samples as discussed above. Our original observations that expression was related to the degree of differentiation has been substantiated in several tumors types and formed the basis for studies of the role of differentiating agents in the expression of Pgp. These studies are being pursued with an eye to understanding further how Pgp function is modulated and also as a means of enhancing the efficacy of the natural products.
5. Active collaborations with David Poplack of the Pediatric Branch have been fruitful in the beginning to understand the role of Pgp in clinical drug resistance. Our findings that Pgp is not expressed in untreated tumors, but can be found in high levels in multiply relapsed patients now form the basis for current efforts to further examine this in a wider group of patients using both in-situ hybridization as well as taking advantage of the polymerase chain reaction. Eventually, this information will be valuable in designing studies in relapsed leukemia with an eye to incorporating the knowledge gained in these studies to initial therapy.



6. Recognizing that Pgp would not explain all clinical resistance and aware of the fact that selection with natural products nearly always results in the selection of cells which overexpress Pgp, we began a selection over two years ago of an MCF-7 cell line with adriamycin and verapamil in order to identify other mechanisms of resistance. The inclusion of verapamil was designed to negate the advantage of Pgp overexpression and thus encourage other mechanisms of resistance. This strategy proved successful and we have now identified a novel membrane protein that it associated with adriamycin resistance and has been found in high levels in clinical samples obtained from patients who relapsed following adriamycin therapy. These studies have utilized polyclonal antibodies raised in rabbits against gel purified protein. All previously described mechanisms of drug resistance have been excluded and the results have been confirmed in independent selections and in selections performed with cell populations selected for low levels of resistance. Current efforts are directed at identifying the protein and determining its primary structure with the aid of molecular cloning techniques. The protein is located on the surface of the cell and has antigenic determinants exposed. Attempts will be starting to obtain monoclonal antibodies directed against this protein. In addition, studies are ongoing to identify other mechanisms of resistance not previously identified. Selections of a colon carcinoma cell line have been identified another mechanism of adriamycin resistance which will be pursued.

7. In the past, we had a very active interest in cisplatin resistance but with the departure of our collaborators from the NIH, these efforts were scaled back considerably. We have now begun once again to direct some of our efforts in this direction, encouraged by our success with the adriamycin resistant MCF-7 cell line and the knowledge that protein changes are present in the cisplatin resistant cell which allow for purification and immunization of rabbits. For these studies, we are using five cell lines developed in our laboratory and are continuing their characterization in some detail.

## PUBLICATIONS

Rothenberg ML, Mickley LA, Balis FM, Cole D, Gillespie A, Poplack DG, Fojo AT. Modulation of the mdr-1/P-170 gene and the dihydrofolate reductase gene in patients with acute lymphoblastic leukemia. *Blood*, in press.

Mickley LA, Bates SE, Richert ND, Rosen N, Fojo AT. Modulation of the expression of a multi-drug resistance gene (mdr/P170) by differentiating agents. *J Biol Chem*, in press.

Bates SE, Mickley, LA, Richert N, Rudick J, Fojo AT. Expression of a drug resistance gene in human neuroblastoma cell lines: Modulation by retinoic acid-induced differentiation. *Mol Cell Biol*, in press.

Fojo AT. Multidrug resistance in tissue culture and human tissues. In: Magrath I, ed. *New directions in cancer treatment*. Berlin: Springer-Verlag, 1989;216-26.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 03024-20 NMOB																												
PERIOD COVERED <u>October 1, 1988 to September 30, 1989</u>																														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Treatment of Extensive Stage Small Cell Lung Cancer</u>																														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)																														
PI: Daniel C. Ihde, MD Others: Adi F. Gazdar, MD R. Ilona Linnoila, MD Michael J. Anderson MD John D. Minna, M.D. Herbert K. Oie, Ph.D. Edward K. Russell	Prof Med NCI-USUHS Senior Investigator Senior Investigator Head, Oncology Branch Chief, NCI-NMOB Microbiologist Chemist	NCI-NMOB NCI-NMOB NCI-NMOB NNMC NCI-NMOB NCI-NMOB NCI-NMOB																												
COOPERATING UNITS (if any) <u>Radiation Oncology Branch; Biostatistics &amp; Data Management Section; Surgical Oncology Division and Hematopathology Branch, National Naval Medical Center, Bethesda.</u>																														
LAB/BRANCH <u>NCI-Navy Medical Oncology Branch</u>																														
SECTION <u>Clinical Investigations</u>																														
INSTITUTE AND LOCATION <u>NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814</u>																														
TOTAL MAN-YEARS: <u>5</u>	PROFESSIONAL: <u>2</u>	OTHER: <u>3</u>																												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Although a dose-response curve clearly exists for alkylating agents in the initial chemotherapy of small cell lung cancer, the therapeutic benefit of higher than standard doses of the more recently introduced regimen of etoposide/cisplatin (VP16/PLAT) is uncertain. We randomized at least partially ambulatory patients with extensive stage SCLC and and without major organ dysfunction to received either VP16 80 mg/m squared Days 1-5 q 3 wks or VP16 80 mg/m squared Days 1-3 + PLAT 80 mg/m squared Day 6 l q 3 wks for the first 6 wks of therapy. Nonambulatory patients and those with organ dysfunction were assigned standard dose treatment. All patients received the standard dose regimen during wks 7-12. From wks 13-24, patients in complete response (CR) continued standard dose VP16/PLAT, while all other patients received a new 3-drug regimen that led to further improvement in response in only 4 cases. CR's were given prophylactic cranial irradiation. Eighty-eight patients have been entered (69 of whom were randomized). With a median follow-up of 40 mos, preliminary results are:</p> <table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th></th> <th>N</th> <th>CR</th> <th>CR+PR</th> <th>Med Surv</th> <th>Nadir WBC</th> <th>Nadir Plt</th> </tr> </thead> <tbody> <tr> <td>High</td> <td>33</td> <td>24%</td> <td>82%</td> <td>12 mos</td> <td>1,700</td> <td>52,000</td> </tr> <tr> <td>Standard</td> <td>36</td> <td>25%</td> <td>83%</td> <td>11 mos</td> <td>2,600</td> <td>178,000</td> </tr> <tr> <td>Nonrand</td> <td>19</td> <td>5%</td> <td>74%</td> <td>6 mos</td> <td>1,800</td> <td>89,000</td> </tr> </tbody> </table> <p>CR rates (p=1.00) and survival (p=0.92) were similar in patients randomized to high and standard dose therapy. There were 2 treatment-related deaths in the high and one in the standard dose arm. We conclude 1) standard dose VP16/PLAT is at least as active as any regimen we have ever utilized for for extensive stage SCLC and produces only modest myelotoxicity, and 2) there is no evidence of superior efficacy when planned drug doses are increased by 67% during the first 6 wks.</p>				N	CR	CR+PR	Med Surv	Nadir WBC	Nadir Plt	High	33	24%	82%	12 mos	1,700	52,000	Standard	36	25%	83%	11 mos	2,600	178,000	Nonrand	19	5%	74%	6 mos	1,800	89,000
	N	CR	CR+PR	Med Surv	Nadir WBC	Nadir Plt																								
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Standard	36	25%	83%	11 mos	2,600	178,000																								
Nonrand	19	5%	74%	6 mos	1,800	89,000																								

## PROJECT DESCRIPTION

## Treatment of Extensive Stage Small Cell Lung Cancer

Professional Staff:

PI:	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	R. Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
	Michael J. Anderson MD	Head, Oncology Branch	NNMC
	John D. Minna, MD	Chief, NCI-NMOB	NCI-NMOB
	Herbert K. Oie, PhD	Microbiologist	NCI-NMOB
	Edward K. Russell	Chemist	NCI-NMOB

Collaborating Branches:

Eli Glatstein and Jane Grayson, Radiation Oncology Branch; Seth M. Steinberg, Biostatistics & Data Management Section; Bimal Ghosh, Surgical Oncology Division, National Naval Medical Center, Bethesda; James Cotelingham, Hematopathology Branch, National Naval Medical Center, Bethesda.

Dr. Ihde has spent six weeks as an attending physician in the Medicine Branch, Clinical Center, where he was responsible for the care of AIDS and other HIV-infected patients on NCI protocols.

Objectives, Rationale, and Background:

This trial has several objectives. We wished to determine in a prospective randomized fashion whether high doses of etoposide (VP16) and cisplatin (PLAT) given during a six-week induction period would produce higher complete response rates and better survival than standard doses of the same drugs in patients with extensive stage small cell lung cancer (SCLC). We also wished to assess the feasibility and value of individualized chemotherapy selection based upon in vitro drug testing of tumor cell lines derived from pre-treatment patient tumor specimens. Objectives of this portion of the study were to determine the frequency with which tumor-containing specimens can be obtained from unselected patients with extensive stage SCLC, the frequency of successful cell culture and drug sensitivity testing, the degree of heterogeneity of drug sensitivity among different cell lines the correlation between in vitro drug sensitivity and clinical response, and the clinical utility of individualized drug selection based upon in vitro data.

The introduction of combination chemotherapy into the management of SCLC has led to four- to five-fold improvement in median survival and five-year disease-free survival in a small fraction of patients. Although median survival is improved to approximately the same degree compared to untreated patients in limited stage and extensive stage disease, survival of two years or more only rarely occurs in patients with extensive disease, defined as tumor extending beyond the hemi-thorax of origin and the regional lymph nodes. Furthermore, chest irradiation has never been suggested to yield any survival benefit in extensive stage patients. Therefore, virtually all patients with extensive SCLC are suitable subjects for investigational chemotherapy studies.

Methods Employed:

Moderately aggressive chemotherapy which produces leukopenia in the range of 1,000/mcl has been shown to be superior to less intensive treatment that is virtually never associated with leukopenic fever in both randomized and non-randomized studies. However, even more intensive initial (or induction) therapy which is so myelosuppressive that hospitalization of all patients is required has not been demonstrated to provide additional benefit, although randomized studies have not addressed this issue. In most of these studies, the drugs given in very high doses have been to provide additional benefit, although randomized studies have not addressed this issue. In most of these studies, the drugs given in very high doses have been restricted to cyclophosphamide, doxorubicin, and VP16. VP16/PLAT has been shown to be a highly synergistic combination regimen in treatment of murine leukemia and in early studies appears to be as active as most three- or four-drug combinations in patients with SCLC. VP16/PLAT is also more active than VP16 alone as a salvage regimen in this tumor. PLAT in higher than conventional doses appears to have increased activity in testicular and perhaps ovarian cancer. Although higher than standard doses of VP16/PLAT have been employed in small studies in SCLC, the issue of dose-response with this combination has not been addressed in a prospective randomized trial. We therefore initiated such a study. The first four patients randomized to the high dose regimen received VP16 120mg/m<sup>2</sup> x 5 and PLAT 40mg/m<sup>2</sup> x 5. Two died of infection before Day 21 without recovery from myelosuppression, and the doses of drugs on the high dose arm were subsequently reduced to VP16 80mg/m<sup>2</sup> x 5 and PLAT 27mg/m<sup>2</sup> x 5. Throughout the trial, doses on the standard arm have been VP16 80mg/m<sup>2</sup> x 3 and PLAT 80mg/m<sup>2</sup> x 1. Since a significant minority of extensive stage SCLC patients are not candidates for a very myelosuppressive regimens, such patients (deemed "poor risk") are not randomized but rather assigned to standard dose therapy.

For the past 10 years, the human tumor stem cell assay of Hamburger and Salmon has been most commonly employed for in vitro drug testing of human cancer. In applying this test to fresh tumor specimens from our SCLC patients, however, we found that sufficient tumor colonies for adequate in vitro testing of even a single drug were present only 23% of the time. Clearly, different approaches were needed to apply in vitro drug testing to a large fraction of patients. Since our laboratory has considerable experience in establishing permanent cell lines of SCLC, we decided to utilize cell lines rather than fresh tumors for drug testing. Compared to fresh tumors, cell lines provide tumor cells that are free of contaminating stromal cells and can be subjected to repeated testing. The time from specimen procurement to assay results, however, is delayed.

A modification of the Weisenthal dye exclusion assay was employed for drug testing because the assay is technically simple, does not require a single cell suspension, can be completed in four days, and can be applied to many tumors and most cell lines. Reading the assay, however is labor intensive and subjective and can be confounded by cell clumping.

Major Findings:

Eighty-eight patients have been entered. Median follow-up from time of patient entry is approximately 40 months. Nineteen of the 88 patients were assigned standard dose therapy because of poor performance status, brain, lung or cardiac dysfunction, or refusal to be randomized. The remaining 69 were randomized to receive high or standard dose VP16/PLAT for the first 6 weeks of therapy.

On the high dose arm, 27 (82%) of 33 have responded to therapy, including 8 (24%) complete responders, and actuarial median survival is 12 months. On the standard dose arm, 30 (83%) of 36 patients responded, including 9 (25%) complete responders, and actuarial median survival is 10 months. There is no significant difference between the two groups in complete response rate ( $p = 1.00$ ) or overall survival by the logrank test ( $p = 0.92$ ). As expected, the response rate (5% complete, 74% complete plus partial) and survival (actuarial median 6 months) are inferior in patients judged not suitable for randomization. Among all 88 patients, performance status and number of distant organ systems involved with metastatic disease (0-2 vs. 3-7) are significant predictors of survival ( $p < 0.001$  and  $p = 0.078$ , respectively).

Hematologic toxicity is significantly worse on the high dose induction program (median nadir WBC count 1,700/mcl and platelet count 52,000/mcl) compared with the standard dose induction (median nadirs 2,600 and 178,000, respectively). Among the poor risk nonrandomized patients, median nadir WBC count has been 1,800/mcl and median nadir platelet count, 89,000/mcl. There have been six treatment-related deaths, all due to myelosuppression and infection, two on the high dose arm prior to lowering of the drug doses, one on the standard dose arm and three in poor risk patients assigned standard dose therapy. Although only 36 patients have been treated, the standard dose regimen yields results at least as good as our historical experience in good risk extensive stage SCLC with considerably less hematologic toxicity, suggesting it may have a superior therapeutic index.

A total of 141 pre-treatment staging specimens have been submitted for cell culture from the first 80 patients (1.8/patient). Seventy-eight specimens (55%) contained tumor cells. Twenty-eight cell lines, defined as sufficient in vitro amplification of tumor cell number to allow testing of multiple drugs in duplicate at three concentrations, have been obtained. The largest numbers of positive specimens and cell lines were derived from bone marrow, peripheral lymph nodes, and pleural effusions. Procurement of only five specimens required administration of general anesthesia, but three of these five procedures were performed for diagnostic purposes. Among the 80 patients with a minimum six-month follow-up, at least one staging specimen reached the cell biology laboratory in 79 (99%), and a tumor-containing specimen was procured from 60 (75%) of these previously untreated patients. A cell line was obtained from 26 (33%), or 43% of patients from whom a tumor-containing specimen was available. In addition, tumor-containing specimens have been obtained from 17 of these patients after tumor progression on chemotherapy, and a cell line has been successfully grown from eight.

Actuarial median survival of patients from whom a tumor cell line was successfully grown, patients from whom a tumor-containing specimen was obtained but did not grow in vitro, and patients from whom no tumor-containing specimen could be procured was 7, 11, and 16 months respectively. Patients with no tumor specimen had superior survival by the logrank test ( $p < 0.01$ ). The survival of patients whose tumor specimens were or were not successfully cultured was not significantly different ( $p = 0.55$ ). Thus, whether a patient had sufficient tumor dissemination that a biopsy specimen could be relatively easily obtained was of greater prognostic import than whether a cell line could be established from a positive biopsy specimen.

In vitro drug testing has been completed on tumor cell lines derived from 22 previously untreated SCLC patients. In vitro drug sensitivity of these cell lines correlated extremely well with response to therapy to VP16/PLAT. In 14/15 (93%) lines from patients with complete or partial response at 12-week restaging, two or more drugs were "active." Sensitivity patterns were strikingly different in the six lines from patients who never responded to VP16/PLAT or had progressed by Week 12. In none of these lines were two or more "active" drugs identified, and only 2/38 (5%) of individual drug assays indicated "activity." P values for these differences were 0.001 and  $< 0.0001$ , respectively. For each of the seven drugs considered individually, lines from responding patients always exhibited a lower mean cell survival at the reference concentration than lines from non-responding patients. Evaluation of these differences with the 2-sample rank yielded p values of less than 0.05 for VP16, doxorubicin, vincristine, and mechlorethamine, and less than 0.10 for methotrexate.

Complete response rates to the first chemotherapy regimen given after VP16/PLAT were compared in patients receiving an "in vitro best regimen" based on in vitro drug testing, or in those receiving vincristine/doxorubicin/cyclophosphamide (VAC) when in vitro drug testing results were not available for whatever reason. Thirty-five patients were treated with VAC after failure to achieve complete response by Week 13, and eight after relapse from complete response induced by VP16/PLAT. In these 43 patients, there were three complete responses (7%). Among the 16 patients who received their "in vitro best regimen," 13 had failed to achieve complete response at Week 13, and three had relapsed. Four patients (25%) attained complete response to their chemotherapy program based on in vitro drug testing ( $p = 0.16$ , Fisher's exact test).

#### Significance to Biomedical Research and the Program of the Institute:

Thus far, there is no indication from this study that a high dose regimen of VP16/PLAT (67% higher doses of each drug, 46% higher doses/unit time actually administered) is in any way superior to standard doses of this two-drug regimen. On the other hand, the standard dose program is well tolerated and may be as effective as any other SCLC regimen, based on this data and that of others. Given the low complete response rate to any of the drug programs given to partial or non-responders at Week 13, it is likely that most or all of the survival benefit our patients received from therapy was produced solely by VP16/PLAT.

The interim results of this trial serve to emphasize several problems that arise in implementing a program of individualized chemotherapy selection with our current technology and study design. First, procurement of tumor specimens, establishment of cell lines, and drug testing are extremely labor intensive and time consuming. More efficient assay techniques and better understanding of the relationship between in vitro and in vivo pharmacokinetics would be valuable. Second, drug testing has been possible in only one-third of patients, and improved methods of cell culture are still needed. We believe these interim results justify the more frequent employment of major surgical procedures to procure larger, more rapidly grown tumor specimens in good risk consenting patients, and have already begun such a program in limited stage patients, who would be expected to more frequently be able to tolerate elective general anesthesia. And third, with the time required to establish and perform drug testing on cell lines, treatment based on in vitro testing can often be given only 10 to 12 weeks after a tumor specimen is obtained and may not be relevant to the in vivo drug sensitivity pattern present in residual tumor cells present at that time. Procurement of larger tumor specimens could help to alleviate this problem and allow more rapid drug testing and quicker administration of "individualized" chemotherapy.

#### Proposed Course:

In a statistical analysis done one month ago, when 69 patients with follow-up had been randomized, 95% confidence limits for differences in 12-month survival ranged from favoring the high dose arm by as much as 32% to favoring the standard dose arm by as much as 19%. We plan to continue accrual to this study until the pre-planned number of 90 patients, which would allow detection of a doubling of complete response rate or a 50% increase in median survival, have been randomized.

Despite these problems and the preliminary nature of our results, we believe several conclusions are justified. First, results of drug sensitivity testing of tumor cell lines are highly correlated with response to initial chemotherapy. Second, preliminary results utilizing in vitro drug testing for individualized selection of chemotherapy regimens suggest modest potential for therapeutic benefit. Third, the close correspondence between in vitro and in vivo response to drugs provides justification for the use of human cancer cell lines in screening for new chemotherapeutic agents. And finally, the availability of multiple SCLC tumor cell lines from patients whose clinical course is well characterized, including some paired lines from patients before and after in vivo chemotherapy, may prove useful in helping to elucidate the basis for drug resistance and other biologic properties of this tumor.

#### Publications:

1. Ihde DC, Johnson BE, Mulshine JL, Sausville EA, Veach SR, Steinberg SM, Edison M, Lesar M, Minna MD. Randomized trial of high vs. standard dose etoposide and cisplatin in extensive stage small cell lung cancer. Lung Cancer 1988;4(suppl):A103. Presented at Fifth World Conference on Lung Cancer of the International Association for the Study of Lung Cancer, Interlaken, Switzerland, 1988.



2. Gazdar AF, Russell EK, Oie HK, Steinberg S, Ghosh B, Linnoila RI, Minna JD, Ihde DC. Extensive disease small cell lung cancer: A prospective trial of chemotherapy based on in vitro drug sensitivity testing. *Adv Biosciences* 1988;72:173-176.
3. Ihde DC, Russell E, Oie H, Linnoila RI, Steinberg S, Ghosh B, Cotelingham J, Minna JD, Gazdar AF. In vivo drug sensitivity testing results correlate with chemotherapy response and survival in extensive small cell lung cancer. *Proc Am Soc Clin Oncol* 1989;8:228. Presented at 25th Annual Meeting of American Society of Clinical Oncology, San Francisco, 1989.
4. Gazdar AF, Tsai CM, Park JG, Ihde DC, Mulshine JL, Carmichael J, Mitchell JB, Minna JD. In vitro assays for predicting clinical response in human lung cancer. In Chapman JD, Peters LJ, Withers HR, eds. *Prediction of Tumor Treatment Response*. New York: Pergamon Press, 1989;175-186.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 06575-14 NMOB
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular pathogenesis and HIV-like retroviruses in the study of lung cancer		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: John D. Minna, MD                      Branch Chief                      NCI-NMOB Others: All NCI-NMOB		
M. Nau	Chemist	R. Osborne, MD      Guest Res (EORTC)
J. Fedorko	Microbiologist	F. Thomas, MD      Guest Res (EORTC)
R. Maneckjee, MD	Guest Res	J. Schuette, MD      Guest Res (EORTC)
T. Takahishi, MD	Fogarty Fellow	I. Chiba, MD      Fogarty Fellow
J. Viallet, MD	Instructor Med NCI-USUHS	
COOPERATING UNITS (if any) H. & W. Nash; H. Brauch, B. Zbar & G. Sithanandam, U. Rapp, FCRF, NCI; J. Whang-Peng, Med Branch, W.E.C. Bradley, Institut du Cancer de Montreal; J. Horowitz, R. Weinberg, Whitehead Institute, MIT; N. Ikegaki & R.H. Kennett, Univ Penn; E. Sausville, MD, Lombardi Cancer Center, Georgetown Univ Hosp.		
LAB/BRANCH NCI-Navy Medical Oncology Branch		
SECTION Genetics, Molecular Biology and Immunology		
INSTITUTE AND LOCATION NCI, COP, DCT, Naval Hospital, Bethesda, MD 20814		
TOTAL MAN-YEARS: 9.5	PROFESSIONAL: 9.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )		
<p>The objective of this work is to identify and characterize the genetic changes (somatic and constitutional) and other deranged mechanisms (such as those involved in growth factors, their receptors and signal transduction) leading to the pathogenesis of lung cancer and to use this information to develop new methods to prevent, diagnose and treat this disease. This work has uncovered abnormalities in the dominantly acting oncogenes and in the more recently described tumor suppressor or recessive (chromosomal deletion) oncogenes including those at 3p, the retinoblastoma gene, and the p53 gene. Most recently we have begun a search for an HIV like retrovirus in the pathogenesis of lung cancer with analogy to the putative retrovirus causing ovine pulmonary carcinomatosis. Growth factor and receptor work has demonstrated the presence of receptors for opioid peptides and nicotine on all classes of lung cancer cells as well as the endogenous production of opioids. These observations lead to new hypotheses concerning the pathogenesis and treatment of lung cancer, particularly with regard to the role of nicotine in cigarette smoke.</p>		

## PROJECT DESCRIPTION

## Oncogenes, Tumor Suppressor Genes, Growth Factors/Receptors, &amp; HIV-like Retroviruses in the Pathogenesis of Lung Cancer

PI: John D. Minna, MD	Branch Chief	NCI-NMOB
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Collaborating NCI-NMOB Branch Personnel:

M. Birrer, MD, PhD, Asst Prof Med, USUHS; F. Kaye, MD, Asst Prof Med USUHS; S. Segal, PhD, Assoc Prof Med, USUHS; I. Linnoila, MD, Senior Pathologist; J. Broers, PhD, Guest Researcher; A. Gazdar, MD, Senior Investigator

Dominantly Acting Oncogenes:

Currently we are studying the role of transcription factors in lung cancer cells including members of the jun family (c-jun, jun-B, jun-D), as well as the proto-oncogene c-fos. We have found deregulated expression of some jun members with loss of control of normal regulatory signals (such as serum and phorbol esters). However, no mutations in the coding regions of the genes have been noted. We demonstrated for the first time, that deregulated expression of normal jun members in concert with a mutated ras gene can transform normal rat embryo cells to malignancy. Because of the relationship of these transcription factors to stimulation by tumor promoters, these findings create the scenario where normal lung and lung cancer cells appear to be in a state similar to chronic tumor promotion. (This work in lung cancer was primarily conducted by Drs. J. Schuette, J. Viallet, and M. Nau)

Expression of the Jun Family of Transcription Factors/Proto-oncogenes in Lung Cancer:

Due to the probable role of tumor promoters in cigarette smoke, and the highly invasive and metastatic nature of lung cancer, we investigated the expression of jun family members in human lung cancer (Schuette, 1988). In Northern blot studies of a large number of tumor cell lines representing all histologic types of lung cancer we found that c-jun (also referred to as jun-A) was expressed to very high levels in about one quarter of the cells, levels much higher than those seen in HeLa cells, placental, or normal liver RNA. Of interest, normal lung expressed c-jun to high levels as well. In contrast, we found jun-B to be expressed to high levels in all of the tumor cell lines, as well as in normal lung. All of the lines expressed only low levels of c-fos mRNA. In studies of rat tissues we also found that c-jun and jun-B were expressed in large amounts in normal lung.

We searched for reasons to account for the high level expression of c-jun (in some cases) and jun-B mRNA in the lung cancer lines. We found no evidence of DNA amplification or rearrangement, and no alteration in mRNA half life (with short half lives of approximately 30 min) suggesting transcriptional activation as the responsible mechanism. Because v-jun has suffered a mutation in the 5' region of the gene, we sequenced one of the cDNAs for c-jun from a small cell lung cancer line and found no mutation in the coding sequence. We conclude from these studies that both normal lung and lung cancer cells express high levels of jun family member mRNAs and, at least in the case of the lung cancer cells, this is related to primary transcription of the genes. Of interest, c-jun appears to positively autoregulate its own expression, and thus, it is possible this autoregulation is taking place in lung cancer cells. (Studies by Drs. Viallet, Schuette, Thomas, Nau)

#### Studies on the Regulation of Expression of Transcription Factors in Lung Cancer:

The regulation of expression of transcription factors including jun family members, the proto-oncogene c-fos, and myc family members has been studied in lung cancer cells after stimulation with phorbol esters, serum, and growth factors. These studies have revealed deregulation, constitutive expression of c-jun and myc family members (the latter associated with DNA or transcriptional changes) while regulation of c-fos remains intact. In addition, while c-myc, N-myc, and L-myc, when associated with amplification, rearrangement or loss of transcriptional attenuation have loss responsiveness to these stimuli, low level c-myc expression retains this response. Overall, these results suggest a fundamental disruption in signal transduction pathways in lung cancer cells. (Studies by Drs. J. Viallet and J. Schuette in collaboration with Dr. E. Sausville, Lombardi Cancer Center, Georgetown Univ. Hospital).

While v-jun can cause tumors in chickens, the transforming activity of the normal cellular homologue, c-jun (jun-A) was unknown. To explore this, we placed a normal human c-jun (one, a cDNA containing the entire open reading frame and another, a human genomic clone from normal DNA) under the control of retroviral promoters and transfected these constructs into normal rat embryo cells alone and with other oncogenes. We found no transformed foci with the c-jun constructs alone or when co-transfected with myc family members (c-myc and L-myc), but found substantial numbers of foci when co-transfected with an activated c-Ha-ras oncogene. These foci were picked and continuous cell lines were established in about 40% of cases. These cell lines would form colonies in soft agar, tumors in athymic nude mice, and they expressed substantial levels of c-jun compared to a very low level in the non-transformed rat embryo cells. We also transfected the constructs into the already immortalized Rat-1a cell line which is susceptible to transformation with single oncogenes. In this case we found that c-jun, by itself under the control of the retroviral LTRs, could transform the Rat-1a cells to malignancy as measured by colony formation in soft agar and formation of tumors in nude mice. However, there were only slight morphologic changes induced in the Rat-1a cells. We also studied the effect of the tumor promoter phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), and found that TPA increased the number of rat embryo cell foci 4 fold. From all of these studies, we conclude that deregulated expression of an unmutated c-jun gene can participate in transformation in cooperation with a mutated ras

gene. However, because only a fraction of the transformed foci can give rise to tumorigenic immortalized lines, other events must also have occurred. (Studies by Dr. Schuette in collaboration with Dr. Birrer)

After demonstrating that deregulated expression of c-jun in cooperation with an activated c-Ha-ras gene can transform primary rat embryo cells (RECs), we studied another member of the jun family, jun-B, and found that it also exhibits transforming activity although to a much lower degree than c-jun. The transforming potential of c-jun and jun-B, in cooperation with ras, can be significantly augmented by phorbol esters and/or cotransfection and deregulated expression of an unmutated human c-fos gene. The relative increase in focus formation by cotransfection of c-fos was higher for jun-B/ras than for c-jun/ras. When c-jun, jun-B and ras were cotransfected into RECs, no significant decrease in the number of foci was observed as compared to the number of foci induced by c-jun and ras alone. When transiently expressed in undifferentiated F9 teratocarcinoma cells, jun-B shows comparable transactivation of an AP-1 site containing reporter gene construct as c-jun. However, cotransfection of c-fos increases transactivation by jun-B to a higher degree than observed with c-jun plus fos. These observations demonstrate differences for jun-B and c-jun with respect to their ability for malignant cell transformation and suggest a differential role of c-fos for its interaction with c-jun and jun-B. (Studies by Drs. Schuette, Nau, Fedorko, Viallet, in collaboration with Dr. Segal)

### Conclusions:

Taken together, the studies in lung cancer cells and in rat embryo cells indicate a scenario in lung cancer cells of deregulated expression of the jun family of transcription factors analogous to that occurring in other cells after growth factor or phorbol ester stimulation. Because such deregulated expression can readily cooperate with ras oncogene mutations in rat embryo cells, it would appear capable of playing a significant role in the pathogenesis of lung cancer. Whether this role becomes evident in the initial transformation of the bronchial epithelium or favors metastatic behavior through stimulation of transcription of genes such as collagenase or stromelysin, remains to be determined. In addition, the stimulation of transcription of genes such as metallothionein IIA could play a role in resistance to chemotherapy. One unexplained feature is the high level of jun expression in RNA isolated from normal lung tissue. This source of RNA represents a mixture of cells, including cells migrating through the lung, and the actual expression in normal bronchial epithelium will have to be studied by techniques such as in situ hybridization. However, if one assumes that the normal bronchial epithelium expresses high levels of jun family members, one possibility is that these factors stimulate the transcription of genes related not only to growth but also to normal bronchial epithelium function and/or differentiation. Thus, another possible scenario is that the chromosome deletion mutations to be described shortly, release the bronchial epithelium from growth control or differentiation signals that would be triggered normally by genes transcribed by Jun. In fact, one could imagine that there would be a strong selection for the inactivation of such genes.

Studies of the myc Proto-oncogenes and their Protein Products in Lung Cancer:

We have been working to characterize the protein products of the L-myc oncogene first found expressed in a deregulated manner in small cell lung cancer (SCLC). For detailed studies of the translation of the gene used in *in vitro* mutagenesis (please see the annual report of Drs. Birrer and Dosaka). We first showed the production of L-myc peptides using anti-peptide antisera prepared against the first myc homology box and the predicted L- or N-myc amino acid sequence. These studies showed the expression of phosphorylated nuclear proteins of relatively short half life for c-myc, N-myc, or L-myc in different SCLC cell lines. In subsequent studies done in collaboration with Drs. N. Ikegaki and R.H. Kennett (Dept of Human Genetics, Univ of Pennsylvania) we have found the human L-myc gene is expressed as two forms of protein in SCLC cell lines using monoclonal antibodies directed against the two myc homology box sequences. These antibodies react with two groups of polypeptides of apparent masses of 60, 61 and 66 kd (the long forms), and 34 and 37 kd (the short forms) in SCLC cells expressing L-myc transcripts. The long form L-myc proteins are associated with the nuclear fraction of the cells. The short form L-myc proteins are present in the cytoplasmic fraction, though diffusion of the short forms from the nucleus during cell fractionation cannot be ruled out. The half-life of the long form polypeptides is 45-90 min. The short form polypeptides have a half-life of 120-180 min. The L-myc protein is not detectable in mitotic cells, suggesting that the L-myc protein expression is tightly regulated during the cell cycle.

Studies of the raf Proto-oncogene in Lung Cancer:

The c-raf-1 proto-oncogene is located in chromosome region 3p25 and frequently shows allele loss in lung cancer. Thus, it is of great interest to characterize its status in lung cancer cells. These studies were done in collaboration with G. Sithanandam and U. Rapp of the Laboratory of Viral Carcinogenesis, FCRF, NCI. Using restriction site polymorphisms (RFLPs) located within the c-raf-1 locus, we examined DNA from 84 human lung carcinomas. In an analysis of 11 paired (normal versus tumor) SCLC DNA samples, all five informative cases showed loss of heterozygosity at this locus in the corresponding tumor sample. Analysis of 73 unpaired lung carcinoma DNAs showed that out of 31 non-SCLC samples, 18% were heterozygous for the BglII polymorphism and 23% showed heterozygosity with TaqI. However, all of the 42 SCLC samples were homozygous for both of these RFLPs. This striking loss of heterozygosity at the c-raf-1 locus in SCLC indicates that one allele of c-raf-1 is deleted in SCLC. The kinase activity of the c-raf protein appears to be constitutively activated in these cells. Whether this apparent activation results from genetic or epigenetic events is under investigation. (Studies by Dr. Viallet in collaboration with Drs. Linnoila and Broers and in collaboration with G. Sithanandam and U. Rapp of the Laboratory of Viral Carcinogenesis, FCRF, NCI)

Tumor Suppressor Genes (Recessive Oncogenes) in Lung Cancer:

Over the past 2 years, lung cancer cells were found to exhibit genetic abnormalities of many potential recessive oncogenes. These ongoing studies have identified abnormalities in the retinoblastoma (rb) gene (chromosome region 13q14) in potentially all SCLC and several non-small cell lung cancers (non-SCLC), and

mutations in p53 (chromosome region 17p13) in over 50% of all lung cancer types. Most recently, SCLC mutants of the *rb* gene have been described that give aberrant protein forms. (In collaboration with Drs. Horowitz and Weinberg, MIT)

In addition, chromosome and RFLP studies have identified lesions on multiple other chromosomes including regions 1p, 1q, 5q, 11p, and 22. A major effort of ours involves trying to identify and isolate the putative recessive oncogene(s) residing in the 3p region.

Because tumor suppressor genes usually require inactivation of both the maternal and paternal chromosomes, this would indicate that as many as 10-15 different genetic lesions have occurred in clinically evident lung cancer. These results have direct bearing on future prevention and prognostic studies and direct the search for early molecular detection of lung cancer and/or the detection of patients exhibiting some of these abnormalities in a premalignant phase. Studies are ongoing to try to "correct" malignancy in lung cancer cells by reintroducing the suppressor genes into lung cancer cells through transfection and retroviral vectors. (Studies by Drs. Takahashi and Nau in collaboration with Drs. Kaye, Birrer, Gazdar and J. Whang-Peng)

#### Demonstration of Allele Loss on Multiple Chromosomes in Lung Cancer Cells, Particularly Regions 3p, 13q, and 17p:

Lung cancer cells have a large number of clonal structural and numerical cytogenetic abnormalities. These include chromosomal deletions with a prominent deletion occurring in chromosome region 3p(14-23). Studies also showed structural and numerical changes involving multiple other chromosomes as well, such as changes on chromosomes 1, 5, 11, 17, and 22. Following the discovery of cytogenetic changes in chromosome 3p, we were interested in proving whether these changes were associated with 3p allele loss in the cancer cells as found in other candidate anti-oncogenes including the *rb* locus. RFLP analysis comparing tumor and normal tissue showed 3p allele loss in nearly all SCLC and 50% or more of non-SCLC. This was also seen by others in Europe and Japan. In addition, our studies and those of Yokota et al. (1987), found allele loss on chromosome 13, while Yokota also found evidence of loss of 17p alleles. In the case of the *rb* inactivation the 13q14 allele loss provided insight into one of the two inactivation lesions, while the allele loss for chromosome regions 3p and 17p suggested a search for new anti-oncogenes in these chromosomal areas. (In collaboration with Drs. J. Whang-Peng, W. Nash, B. Zbar, H. Brauch, W.E.C. Bradley, U. Rapp)

#### Inactivation of the *rb* Gene in Lung Cancer:

In studies of a large number of lung cancer lines for the status of the *rb* gene we found DNA abnormalities in 4/26 SCLC, 3/4 pulmonary carcinoids, and 0/20 non-SCLC. The DNA abnormalities included homozygous and heterozygous deletions of portions of the *rb* gene. In addition, we found absent or trace *rb* mRNA expression in 20/26 (77%) of SCLC, 3/4 carcinoids, and 4/19 (21%) of non-SCLC. In related studies, Yokota et al., (1988) found no immunoprecipitable protein in 9/9 SCLC and 2/9 non-SCLC. The involvement of the more benign pulmonary carcinoids, as well as the inactivation of the *rb* gene, in perhaps all SCLC and some

non-SCLC argues for an early and significant role for this gene in the pathogenesis of lung cancer. It will be particularly interesting to characterize these mutant rb proteins produced by lung cancer cells to look for lesions which give clues to the functional division of the rb protein. (In collaboration with Dr. F. Kaye)

#### Abnormalities and Inactivation of the p53 Gene in Lung Cancer:

Following the discovery of cytogenetic abnormalities of chromosome region 17p allele loss by RFLP analysis in lung cancer cells, the assignment of p53 to this region, and the recent studies indicating that p53 is a candidate anti-oncogene, we examined its structure and expression in lung cancer. In studies of p53 structure and expression in SCLC and non-SCLC, we have found DNA abnormalities including homozygous deletions, mRNA abnormalities, and many examples of point or small mutations occurring in the open reading frame of p53 (Takahashi et al., in preparation). In addition, small mutations were found in tumor cell specimens taken directly from patients without intervening cell culture, while several other tumor cell lines failed to produce p53 mRNA. We conclude that the p53 gene appears to be frequently affected in all types of lung cancer. We note that similar mutations have recently been reported for human colon cancer where 17p allele loss is also seen. (Studies by Drs. Takahashi and Nau in collaboration with Drs. Birrer and Gazdar)

#### Conclusions:

It is clear from the above studies that lung cancers frequently suffer allele loss and inactivation of anti-oncogenes including the rb and p53 genes, as well as the probable inactivation of other genes such as those on chromosome region 3p. In fact, a reanalysis of the British doctors' cigarette smoking data fits a recessive oncogene model well (Moolgavkar, 1989). Obviously, if these genes are acting as recessive anti-oncogenes and two hits are required for inactivation, lung cancer cells will have to suffer many genetic lesions. The number increases even more when one considers those activating the dominantly acting oncogenes.

The number of lesions raises the question of the timing of the mutations. While they could have all occurred during adult life as the result of cigarette smoking, it is also possible that some of them could be inherited in a Mendelian fashion or acquired during embryonic development of the bronchial epithelium as must occur in the developing retina for the first lesion in the rb gene in cases of sporadic retinoblastoma. While lung cancer is not generally considered to be an inherited disease, there are actually several pieces of evidence indicating potential inherited predisposition. In the case of the rb gene, there are patients cured of familial retinoblastoma who have gone on to develop small cell lung cancer (Messmer, 1987; Leonard, 1988). While these are obviously rare occurrences, it is not unreasonable to speculate there could be allelic forms of a mutant rb gene which do not lead to sporadic retinoblastoma, but in the presence of cigarette smoking and gross deletion of a normal allele could lead to the development of small cell lung cancer. Peto has calculated that for a mutant gene with a frequency of 0.3 to 0.5 in the population and with an effect of increasing cancer 50 to 100 fold over the wild type state, a recessive model



would predict siblings of such affected patients to have an incidence of cancer approximately 2-4 fold over the general population (Peto, 1980). In fact, there is a 2-4 fold increased risk of several types of cancer in family members of lung cancer patients (Tokuhata, 1963; Lynch, 1986; Ooi, 1986; Samet, 1986). All of this prompts a study to determine the timing of the development of mutations found in lung cancer. If some of these are inherited or occur during embryonic life, entirely new prevention and screening strategies could be developed.

#### The Role of Opioids and Nicotine in the Pathogenesis of Lung Cancer:

Receptors for the major addictive agents opioids and nicotine were found on all types of lung cancer. The opioids were found to negatively influence lung cancer growth in vitro while nicotine, in many cases, reversed this effect. These findings provide new approaches to therapy and suggest focusing a major effort on the role of nicotine not only as an addictive agent in smoking, but as possibly playing a major role in the pathogenesis of lung cancer. (Studies by Dr. R. Maneckjee)

We find that SCLC and non-SCLC express specific, high affinity ( $K_d = 10^{-9}$ – $10^{-10}$  M) receptors ( $\mu$ ,  $\delta$ ,  $\kappa$ , PCP) for various opioid agonists. Using specific radioligands for the receptor types, we were able to show that both SCLC and non-SCLC express multiple opioid receptors with the major differences between lung cancer types being the expression of the  $\kappa$  subtype in SCLC and PCP receptors in non-small cell lung cancers. These receptors were biologically active since cAMP levels decreased significantly after  $\mu$ ,  $\delta$ , and  $\kappa$  agonist application. The lung cancer cells also expressed immunoreactive opioids (including endorphin, enkephalin, and dynorphin) while exogenously added opioids in the concentration range of 1-100 nM inhibited lung cancer growth in vitro, an effect reversed by the opioid antagonist naloxone. Because of the known physiologic antagonism of nicotine and morphine and the almost universal exposure of patients with lung cancer to nicotine, we assayed for nicotine and  $\alpha$ -bungarotoxin receptors and found specific, high affinity [ $-$ ] [ $^3$ H]-nicotine receptors ( $K_d = 10^{-9}$ ) on both SCLC and non-SCLC but  $\alpha$ -bungarotoxin receptors only on SCLC. Of interest, morphine and the  $\kappa$  agonist U-50,488H displaced  $^3$ H-nicotine from SCLC membranes, and while nicotine alone lowered cAMP levels, the combination of nicotine and opioids either reversed this effect or stimulated cAMP levels. The addition of nicotine (1-100 nM), while having only a slight growth effect alone, reversed opioid inhibition of SCLC but not non-SCLC growth. These results suggest a model where endogenously produced opioids inhibit bronchial epithelium and lung cancer growth while exogenous nicotine relieves lung cancer cells from this inhibition.

#### Search for Toxins and Drugs Which Could Inhibit the Signal Transduction Pathways Activated in Lung Cancer with Their Development for Use in Patient Treatment:

Cholera toxin was found to dramatically inhibit the in vitro growth of many lung cancer cell lines. This was related to the expression of specific glycolipid membrane receptors. Further preclinical studies of this toxin are underway to attempt to bring it to clinical trial in the treatment of lung cancer. (Studies by Dr. J. Viallet in collaboration with Dr. E. Sausville, now at Lombardi Cancer Center).

Search for Retroviruses Related to HIV as a Causative Agent in Human Bronchiol-alveolar and Other Types of Lung Cancer and Attempts to Clone and Characterize the Retrovirus Causing Lung Cancer in Sheep:

These studies are beginning and include a search for reverse transcriptase activity in the supernatant fluids of lung cancer cell lines, screening of lung cancer DNAs with probes from HIV and other primate retroviruses and oligo-nucleotides with preserved sequence between different retrovirus, to look for cross reacting bands, the use of polymerase chain reaction technology, and the inoculation of lung cancer cells into the lungs of new born sheep to attempt to replicate the sheep disease with human cells. This work includes epidemiologic studies in collaboration with the Epidemiology Branch (Drs. Madigan and Mulvihill) as well as pathologic review of lung cancer incidence and the changing epidemiology of bronchiol-alveolar lung cancer. (Studies by Dr. R. Osborne)

Publications:

1. DeGreve J, Battey J, Fedorko J, Birrer M, Evan G, Kaye FJ, Sausville EA, Minna JD. The human L-myc gene encodes multiple nuclear phosphoproteins from alternatively processed mRNAs. *Mol Cell Biol* 1988;8:4381-4388.
2. Whang-Peng J, Lee EC, Minna JD, Abeloff MD, Bradley EC, Young RC, Longo DL. Deletion of 3(p14p23) in secondary erythroleukemia arising in long-term survivors of small cell lung cancer. *J Natl Cancer Inst* 1988;80:1253-1255.
3. Schuette J, Minna JD, Birrer MJ. Deregulated expression of human transcription factor c-jun transforms primary rat embryo cells in cooperation with an activated c-Ha-ras gene and Rat-la cells as a single gene. *Proc Natl Acad Sci USA* 1989;86:2257-2261.
4. Leduc F, Brauch H, Hajj C, Dobrovic A, Kaye F, Gazdar AF, Harbour JW, Pettengill OS, Sorenson GD, van den Berg A, Kok K, Campling B, Paquin F, Bradley WEC, Zbar B, Minna JD, Buys C, Ayoub J. Loss of heterozygosity in a gene coding for a thyroid hormone receptor in lung cancers. *Am J Hum Genetics* 1989;44:282-287.
5. Ikegaki N, Minna JD, Kennett RH. The human L-myc gene is expressed as two forms of protein in small cell lung carcinoma cell lines: Detection by monoclonal antibodies specific to two myc homology box sequences. *EMBO* 1989;8:1793-1799.
6. Miller YE, Minna JD, Gazdar AF. Lack of expression of aminocyclase I in small cell lung cancer: Evidence for inactivation of genes encoded by chromosome 3p. *J Clin Invest* 1989;83:2120-2124.
7. Birrer MJ, Minna JD. Genetic changes in the pathogenesis of lung cancer. *Ann Rev Med* 1989;40:305-317.
8. Sathanandam G, Dean M, Brennscheidt U, Beck T, Gazdar AF, Minna JD, Brauch H, Zbar B, Rapp U. Loss of heterozygosity at the c-raf locus in small cell lung carcinoma. *Oncogene*, In press.

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PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: I.R. Kirsch, MD</td> <td style="width: 33%;">Senior Investigator</td> <td style="width: 33%;">NCI-NMOB</td> </tr> <tr> <td>Others: V. Bertness</td> <td>Biol Lab Tech</td> <td>NCI-NMOB</td> </tr> <tr> <td>K. Nakahara</td> <td>Biologist</td> <td>NCI-NMOB</td> </tr> <tr> <td>G. Begley, MD, PhD</td> <td>Staff Fellow</td> <td>NCI-Metabolism</td> </tr> <tr> <td>P. Aplan, MD</td> <td>Med Staff Fellow</td> <td>NCI-Pediatrics</td> </tr> <tr> <td>M-H. Stern, MD</td> <td>Guest Researcher</td> <td>NCI-NMOB</td> </tr> <tr> <td>S. Lipkowitz</td> <td>Instructor Med NCI-USUHS</td> <td>NCI-NMOB</td> </tr> </table>			PI: I.R. Kirsch, MD	Senior Investigator	NCI-NMOB	Others: V. Bertness	Biol Lab Tech	NCI-NMOB	K. Nakahara	Biologist	NCI-NMOB	G. Begley, MD, PhD	Staff Fellow	NCI-Metabolism	P. Aplan, MD	Med Staff Fellow	NCI-Pediatrics	M-H. Stern, MD	Guest Researcher	NCI-NMOB	S. Lipkowitz	Instructor Med NCI-USUHS	NCI-NMOB
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SECTION Acquired Gene Rearrangements																							
INSTITUTE AND LOCATION NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814																							
TOTAL MAN-YEARS: 6	PROFESSIONAL: 4.5	OTHER 1																					
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>Association of a specific chromosomal abnormality with tumor type is well established and may reflect mechanisms of oncogenesis peculiar to that tumor. Alternatively, it may be that these associations reflect the particular differentiated state of the malignant cell, consistent with the model that rearrangements occur only within chromatin in an "active" configuration.</p> <p>Our focus this year is on abnormalities of human chromosomes 7 and 14 in "normal" and malignant lymphocytes. Previously we identified the formation of T-cell receptor (TCR) alpha chain-immunoglobulin heavy (IgH) chain hybrids as a consequence of the inversion of chromosome 14 in both a T and B cell malignancy. This inversion appeared to be morphologically identical to the occasional inversion of this chromosome in the cells of all normal individuals. A related but distinctive inversion or translocation of chromosome 14 is most likely associated with a particular type of T-cell malignancy, as well as with a clonal proliferation of T-cells in patients with the disease, ataxia-telangiectasia. We have cloned and characterized the chromosome 14 breakpoints from T-cells from a patient with ataxia-telangiectasia and from a cell line derived from a patient with a particular T-cell lymphoma. The proximal breakpoints in both these cases is again (as above) within the TCR alpha locus. The distal breakpoints do not appear to involve IgH, but rather identify a more proximal region, perhaps containing a new growth effecting gene function.</p> <p>. AT patients also carry an increased frequency of inv(7) in their peripheral blood lymphocytes. We cloned and characterized one such abnormality and found it to be an in-frame TCR V<math>\gamma</math>-TCR J<math>\beta</math> fusion somewhat analogous to (and raising the same questions regarding functional meaning) as the hybrid inv(14) discussed above.</p> <p>Finally, our work in the characterization of a t(1;14) from the cells of a patient with stem cell leukemia has led us to the identification of a new gene, "scl", which we believe to be important in the decision of stem cell commitment and differentiation.</p>																							

## PROJECT DESCRIPTION

## Chromosomal Abnormalities that Highlight Regions of Differentiated Activity

Professional Staff:

PI:	Ilan R. Kirsch, MD	Senior Investigator	NCI-NMOB
Others:	V. Bertness	Biol Lab Tech	NCI-NMOB
	K. Nakahara	Biologist	NCI-NMOB
	G. Begley, MD, PhD	Staff Fellow	NCI-Metabolism
	P. Aplan, MD	Med Staff Fellow	NCI-Pediatrics
	M-H. Stern, MD	Guest Researcher	NCI-NMOB
	S. Lipkowitz	Instructor Med	NCI-USUHS
			NCI-NMOB

Objectives:Long Term

1. To define the necessary and/or sufficient features for chromosomal breakage and rejoining in different cell types.
2. To determine if chromosomal breakage disorders, particularly ataxia-telangiectasia, represent an exaggeration of normal events or a novel pathology.
3. To use the occurrence of cell-type specific chromosomal aberrations as an in-road to the exploration of differential gene activation during development.
4. To contribute to the understanding of how gene rearrangements mediated by chromosomal aberrations alter the regulation of the affected loci.

Short Term

To study chromosomal aberrations in hematopoietic cells, particularly:

1. To determine the frequency and cell type distribution of inversions and translocations of human chromosomes 7 and 14 in normal, "pre-malignant", and malignant conditions and explore whether there is evidence for selective or random associations between particular breakpoints and particular transformed or proliferative states.
2. To clone and sequence the breakpoints from "normal" and malignant cells carrying inversions or translocations of chromosomes 7 and 14. To assess from sequence data, if possible, how the chromosomal aberration occurred and what loci were involved.
3. To study the genomic activity of loci involved in translocations and inversions of chromosomes 7 and 14 in corresponding cells in which the chromosomal aberration has or has not occurred, and thereby determine if the aberration has caused deregulation or altered expression of these loci.

4. To continue our studies on the mechanisms of oncogene deregulation as a result of chromosomal rearrangements in lymphoid malignancies.

#### Major Findings:

Previously (see previous annual reports) we have demonstrated that in hemato-poietic cells, chromosomal abnormalities often involve regions of differentiated activity. Using this concept as a predictive and testable model we recently focused on chromosomal abnormalities associated with T cell malignancies, mapped the T cell antigen receptor genes by chromosomal in situ hybridization, and analyzed two cases of inversions of chromosome 14 that involved the T cell receptor alpha chain locus. These inversions had been formed by a site-specific recombination event between a T cell antigen receptor alpha chain joining (J) segment (at 14q11.2) and an immunoglobulin heavy chain variable (V) segment (at 14q32.3). The formation of the hybrid gene, part immunoglobulin, part T cell receptor, provided an attractive model for the generation of lymphoid malignancies. Immunoglobulin (Ig) and T cell receptors (TCR) are, after all, cell surface receptors capable of transducing mitogenic (antigenic) stimuli at the cell surface into cellular proliferative activity. It was conceivable that either because of its function or specificity a hybrid Ig/TCR gene might stimulate an abnormal proliferative response. Observations in certain retroviral transduction systems lent support to this view. Occasionally a retrovirus containing an intact antigen receptor gene can be found associated with certain types of lymphoid malignancies.

Additional support for this concept has now come from our characterization of an inv(7) abnormality found in the peripheral blood lymphocytes (and derived T cell line) from a patient with ataxia-telangiectasia (AT). Such inversions are often seen in PBLs of AT patients and may comprise 1-5% of the PHA stimulated metaphases (see below). Using PCR technology, we have been able to clone and sequence this abnormality from both the patient and cell line. This particular inversion of chromosome 7 has been mediated by a site specific recombination event between a TCR gamma variable segment (located at 7p13) and a TCR beta joining segment (located at 7q35). The joining event occurred in-frame and is transcribed into a hybrid  $\gamma$ - $\beta$  message (the in-frame and transcribed nature of this abnormality is similar to that seen in the inv(14) hybrids described above).

Such inversions of chromosome 7 and 14 are also found in normal individuals at a frequency (by brute force karyotypic analysis) of approximately 1/10,000 cells and 1/1000 cells, respectively. The mono, oligo, or polyclonal nature of these abnormalities in the cells of normal or AT patients is being explored. We are also attempting to get a more definitive assessment of their time frequency and whether they differ in percentage in or out of frame.

While still an attractive model our further studies suggest that the contribution of translocation and/or inversion of chromosomes 7 and 14 to the development of lymphoid malignancy is more complicated than it first appeared. In the lymphoid malignancies which show the clearest association of a translocation or inversion of chromosome 14 the distal chromosomal breakpoint does not seem to involve the Ig heavy chain locus as it had in the two sporadic tumors we had

analyzed above. We have analyzed, in molecular detail, a t(14;14) found in a cell line derived from a patient with a T cell leukemia. The proximal breakpoint is clearly within the TCR alpha chain joining region. The distal (14q32) breakpoint involves a region 3' and centromeric to the IgH locus. Further insight into this issue may again be gained from our studies of material from patients with the disease, ataxia-telangiectasia. AT is a disease of protean manifestations including progressive neurologic deterioration, ocular telangiectasia, immunodeficiency, a tendency to develop chromosomal breaks, and a predisposition to cancer, particularly lymphoid malignancies. The karyotype of PHA stimulated lymphocytes from patients with AT will often show translocations and inversions within and between chromosomes 7 and 14 involving the chromosomal bands to which are localized the TCR alpha beta, gamma, and delta chain. This underscores the fact that in T cells chromosome abnormalities do indeed often involve regions of differentiated activity and highlight the propensity of these patients to develop cell-type specific chromosomal breaks. This may be a unique defect in this disease or an exaggeration of a normal error-prone lymphoid recombination system. This distinction is being investigated in our laboratory at present. Occasionally a lymphoid cell is generated in patients with AT that seems to have a proliferative advantage. These cells almost always carry inversions or translocations of chromosome 14. They can reach a point where they comprise 100% of the PHA stimutable population in the patient and can be shown by Southern blot to represent a single T cell clone. We have identified and analyzed one such t(14;14) from a 32 year old female with AT. Analogous to the T-ALL cell line the proximal breakpoint in this patient's cells was again within the TCR alpha chain J region, the distal breakpoint within a region 3' and centromeric to the IgH locus. The two 14q32 breakpoints analyzed in these two cases have not yet been linked to each other, nor has a new presumptively growth effecting gene yet been identified in this region. We feel these studies are addressing fundamental issue in multi-step carcinogenesis and genomic instability.

We have recently started to analyze two related chromosomal aberrations which share one or the other region with these t(14;14) cases. One is a lymphoid/myeloid (stem cell) cell line carrying a t(1;14) (p32;q11.2) in which there is clear rearrangement of both the TCR alpha and delta loci. We have cloned both rearrangements. The TCR alpha rearrangement shows evidence of a specific TCR delta deleting element presumed to be an important event in lymphoid maturation and functional "capacitation". The TCR delta rearrangement is even more fascinating. It has occurred reciprocally with a region on chromosome 1 that appears to be transcribed differentially in early hematopoietic cells. We have named this putative gene, scl. There is a 4 kb scl transcript seen in cells and cell lines that show no karyotypic abnormality of chromosome 1. In the t(1;14) cells, the transcript is truncated at its 3' end and has formed a fusion transcript with part of the TCR delta locus. Full length cDNA cloning and characterizing of the normal and truncated transcripts are proceeding in our laboratory. We are also studying a tumor sample from a patient with ALL whose tumor cells appear to carry a t(5;14) (q33;q32).

We are focusing on the basic contribution of these loci to the development of lymphoid/myeloid malignancy and the necessary and sufficient factors required for the mechanism of chromosomal breakage and rejoining to operate.

Publications:

1. Davey MP, Bertness V, Nakahara K, Johnson JP, McBride OW, Waldmann TA, Kirsch IR. Juxtaposition of the T-cell receptor  $\alpha$ -chain locus (14q11) and a region (14q32) of potential importance in leukemogenesis by a 14;14 translocation in a patient with T-cell chronic lymphocytic leukemia and ataxia-telangiectasia. *Proc Natl Acad Sci USA* 1988;85:9287-9291.
2. Begley GB, Aplan PA, Davey MP, Nakahara K, Tchorz K, Kurtzberg J, Haynes BF, Hershfield M, Cohen D, Waldmann TA, Kirsch IR. Chromosomal translocation in a human leukemic stem cell line disrupts the TCRD  $\delta$  region and results in a previously unreported fusion transcript. *Proc Natl Acad Sci USA* 1989;86:2031-2035.
3. Begley GB, Aplan PD, Waldmann TA, Kirsch IR. A novel gene, SCL identified by a chromosomal translocation in a hematopoiesis stem-cell leukemia. In Golde D, Clark S, eds. *Hematopoiesis, UCLA Symposia on Molecular and Cellular Biology*. New York: Alan R Liss, 1989.
4. Bertness V, Felix CA, McBride OW, Morgan R, Smith SD, Sandberg A, Kirsch IR. Characterization of the breakpoint of a t(14;14)(q11.2;q32) from the leukemic cells of a patient with T-cell acute lymphoblastic leukemia. *Cancer Genet Cytogenet*, in press.
5. Begley CG, Aplan PA, Davey MP, deVillartay J-P, Cohen DI, Waldmann TA, Kirsch IR. Demonstration of  $\delta$  rec-pseudo J $\alpha$  rearrangement with deletion of the delta locus in a human stem-cell leukemia. *J Exp Med*, in press.
6. Stern M-H, Lipkowitz, S, Aurias A, Griscelli C, Thomas G, Kirsch IR. Inversion of chromosome 7 in ataxia telangiectasia is generated by a rearrangement between T-cell receptor beta and T-cell receptor gamma genes. *Blood*, submitted.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 06581-06 NMOB
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Genetics of Differentiation and Transformation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: W. Michael Kuehl, MD Others: Diana McClinton (term 12/31/88) Cynthia Timblin, PhD Francine Foss, MD Leslie Brents (EOD 10/9/88) Lief Bergsagel, MD	Senior Investigator Microbiologist Staff Fellow Asst Prof Med NCI-USUHS Res Assoc NCI-USUHS Medical Staff Fellow	NCI-NMOB NCI-NMOB NCI-NMOB NCI-NMOB NCI-NMOB NCI-NMOB
COOPERATING UNITS (if any) None		
LAB/BRANCH NCI-Navy Medical Oncology Branch		
SECTION Molecular Biology of Differentiation		
INSTITUTE AND LOCATION NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814		
TOTAL MAN-YEARS: 5.3	PROFESSIONAL: 5.3	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>First, we have demonstrated that the continuous expression of either a <u>c-myc</u> or <u>c-myb</u> transgene blocks terminal differentiation of MEL cells. We have also shown that the late down-regulation of both genes is necessary for chemically induced terminal differentiation, whereas the early down-regulation of these genes does not appear to be essential for differentiation. In addition, we have shown that continuous expression of a <u>c-myc</u> transgene in an IL-3-dependent murine pregranulocyte leukemia cell line results in cells with a variant phenotype, i.e. cells which are unable to differentiate in differentiation medium (i.e. medium in which G- CSF replaces IL-3), but which have an enhanced ability to survive in differentiation medium. These results provide some insight into the cause of differentiation block which is observed in many malignant tumors. Second, we have developed a general method for subtractive cloning by incorporating polymerase chain reaction (PCR) technology into the preparation and analysis of subtractive cDNA libraries. This new technology is being used to identify differentially expressed genes which are important in hematopoietic differentiation.</p>		



## PROJECT DESCRIPTION

## Molecular Genetics of Differentiation and Transformation

Overall Objectives:

1. To clarify the cellular and molecular mechanisms which determine and regulate hematopoietic differentiation.
2. To clarify the relationship between differentiation and malignancy.

Species Studied: Mice and humansA. Role of c-myc and c-myb oncogenes in hematopoietic differentiation (in collaboration with S. Segal)

PI:	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	Diana McClinton (term 12/31/88)	Microbiologist	NCI-NMOB
	Cynthia Timblin, PhD	Staff Fellow	NCI-NMOB
	Francine Foss, MD	Asst Prof Med NCI-USUHS	NCI-NMOB
	Leslie Brents (EOD 10/9/88)	Res Assoc NCI-USUHS	NCI-NMOB
	Lief Bergsagel, MD	Medical Staff Fellow	NCI-NMOB

Endogenous c-myc and c-myb mRNA levels decrease biphasically when murine erythroleukemic (MEL) cells are induced to differentiate with various chemical inducers. Previously we introduced a c-myc construct into MEL cells by stable transfection and determined that continuous expression of c-myc mRNA blocks chemically induced terminal differentiation of MEL cells. Our colleagues (M. Birrer and S. Segal) have recently demonstrated that although L-myc is not expressed in MEL cells, a transfected human L-myc cDNA can substitute for c-myc in blocking chemically induced terminal differentiation. We have demonstrated that continuous expression of a transfected murine c-myb cDNA in MEL cells also blocks chemically induced terminal differentiation.

By transfecting c-myb or c-myc cDNAs under the control of a mouse metallothionein gene into MEL cells, we can rapidly up- or down-regulate the expression of the transgene by increasing or decreasing the amount of ZnCl<sub>2</sub> in the medium. In altering the level of transgene expression during the chemically induced differentiation process, we have been able to demonstrate that the late down-regulation of both c-myc and c-myb is necessary for terminal differentiation. By contrast, it appears that the early down-regulation of these genes is not necessary for the terminal differentiation process. In addition, we have preliminary evidence which suggests that chemically induced MEL cells are at least partially committed to terminal differentiation when either c-myc or c-myb is expressed at high levels. This conclusion is based on the observation that a fraction of the cells complete the terminal differentiation process when ZnCl<sub>2</sub> and the chemical inducer are present several days but then removed together from the culture medium.

Transfection of a c-myc cDNA into an IL3-dependent murine pregranulocyte leukemia cell line results in cells which have lost the ability to terminally

differentiate in differentiation medium (i.e. medium in which G-CSF replaced IL-3), although showing an increased ability to survive in this same medium.

#### Publications:

1. Kuehl WM, Bender TP, Stafford J, McClinton D, Segal S, Dmitrovsky E. Expression and function of the c-myb oncogene during hematopoietic differentiation. Curr Topics Microbiol Immunol 1988;141:318-323.
  2. Bender TP, Catron KM, Kuehl WM, Thompson CB. Sense and anti-sense transcription in the murine c-myb attenuator region. Curr Topics Microbiol Immunol 1988;141:324-329.
  3. McClinton D, Stafford J, Brents L, Bender TP, Kuehl WM. Differentiation of mouse erythroleukemia cells requires the second phase of c-myb down-regulation. Mol Cell Biol, submitted.
- B. Identification of genes involved in differentiation by subtractive cDNA cloning (in collaboration with J Battey, NINDS)

PI: W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others: Cynthia Timblin, PhD	Staff Fellow	NCI-NMOB
Lief Bergsagel, MD	Medical Staff Fellow	NCI-NMOB

We have developed a general method for subtractive cloning incorporating polymerase chain reaction (PCR) technology into the preparation and analysis of subtractive cDNA libraries. This new approach permits the rapid and efficient analysis of differentially expressed genes, including genes which express as little as one copy of mRNA per cell. We have used this novel technology to identify genes which are expressed in a mouse plasmacytoma tumor but not in a mature B lymphoma which secretes immunoglobulin. From screening about 3% of the library, we have identified about 40 genes which are expressed in the plasmacytoma parent but not in the B lymphoma subtractive partner. Surprisingly, only one of these genes is expressed in three unrelated plasmacytomas but no other B cell or pre-B cell lines, although two of these genes are expressed in two of three unrelated plasmacytomas and no other B cell or pre-B cell lines examined. We also find a number of genes which are expressed in three unrelated plasmacytomas and two pre-B cell lines but not in three unrelated B cell lines. This latter class of genes is interesting since there is increasing evidence that human multiple myeloma may be more closely related to pre-B cells than B cells. In addition, we are beginning to define the differences in gene expression between a sporadic Burkitt's lymphoma and a human myeloma cell line. Finally, we are collaborating with Shosh Segal and her group to identify genes which are differentially expressed in induced vs. uninduced MEL cells. Our ultimate goal is to identify and isolate genes which determine and regulate hematopoietic differentiation.

#### Publications:

1. Timblin CR, Battey J, Kuehl WM. The use of PCR technology to prepare and analyze cDNAs differentially expressed in murine plasmacytoma and murine B lymphoma cell lines. In preparation.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 06587-05 NMOB
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gene Rearrangements as Tumor Specific Markers		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:           Ilan R. Kirsch, MD Others:   Carolyn Felix, MD Kenneth Nakahara Nita Seibel, MD Kathryn Tchorz	Senior Investigator Medical Staff Fellow Biologist Guest Researcher SRTP	NCI-NMOB NCI-NMOB NCI-NMOB NCI-NMOB NCI-NMOB
COOPERATING UNITS (if any)		
LAB/BRANCH NCI-Navy Medical Oncology Branch		
SECTION Acquired Gene Rearrangements Section		
INSTITUTE AND LOCATION NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814		
TOTAL MAN-YEARS 2.8	PROFESSIONAL: 2.8	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Differential structural alterations and expression of immunoglobulin (Ig), T cell receptor (TCR), and various growth effecting genes are studied in malignant tumors and their derivative cell types. Studies are carried out to diagnose, classify, and stage lymphoid malignancies via a) Southern and Northern blot analysis, b) RNA-RNA tissue <u>in situ</u> hybridization and c) chromosome <u>in situ</u> hybridization. A. DNA and RNA is extracted from the tumors of patients with acute lymphoblastic leukemia (ALL) of infancy, pre B ALL, T cell ALL, mycosis fungoides, and Sezary syndrome. The structural reconfigurations of DNA around the Ig and TCR loci resulting from the normal functional activation of these loci in these cells provide unique "fingerprints" for identifying clonal populations in the samples and following these populations during the course of treatment. We have shown by these analyses that the above listed lymphoid malignancies each manifest generally distinguishing genotypic patterns reflecting target cell maturation which to a certain extent recapitulates the age incidence of the development of these tumors. B. RNA-RNA tissue <u>in situ</u> hybridization. The expression of individual cells within tissue sections from lymph node biopsies and peripheral blood from patients with lymphoid malignancies have been analyzed with immunoglobulin, T cell receptors, and oncogene probes. This technique refines the analysis of such tissue to the point where the unique gene expression of one cell in hundreds of thousands can be identified. Furthermore we have developed a technique of direct RNA sequencing the allows us to utilize tumor specific gene expression as a tumor specific marker providing a potentially powerful and sensitive means of cancer diagnosis and staging. C. Gene and transcript mapping. We have localized numerous genes of interest to specific regions of human chromosome by <u>in situ</u> hybridization. Refinement of this technique using biotinylated probes is increasing the sensitivity and specificity of our work and allows us to study genotopography in interphase nuclei.		

## PROJECT DESCRIPTION

## Gene Rearrangements as Tumor Specific Markers

Professional Staff:

PI:	Ilan R. Kirsch, MD	Senior Investigator	NCI-NMOB
Others:	Carolyn Felix, MD	Biotech. Fellow	NCI-NMOB
	Kenny Nakahara	Biologist	NCI-NMOB
	Nita Seibel, MD	Guest Researcher	NCI-NMOB
	Kathryn Tchorz	S RTP	NCI-NMOB

Objectives:

1. To develop, master, and refine techniques based on molecular genetics which are of direct current application in the diagnosis, classification, and staging of patients with cancer.
2. To demonstrate the usefulness of these techniques in pilot studies.
3. To promote the adoption of these techniques by service oriented laboratories, and supervise the implementation of such techniques in a standardized quality controlled fashion for comprehensive, prospective, best available therapy protocols and epidemiological studies.
4. To use the data generated in the pilot and/or comprehensive analyses as a resource of information and a bank of material for further studies to be carried out in basic research laboratories.

Methods Employed:

As a result of the normal functionally activating recombination events that occur in B and T lymphocytes, the structural configuration of the DNA around the immunoglobulin or T cell receptor loci is irreversibly altered in each differentiated cell. Because of the unique nature of the antigen-receptor molecules being produced, the VJ or VDJ recombinatorial event (and DNA configuration) in one cell making one antibody or T cell receptor will be distinct from a cell making an antibody or T cell receptor with a different antigen-binding capacity. Thus, by the necessity of its role in the immune response, every lymphocyte beyond a certain stage in its development carries within it a unique molecular DNA "fingerprint".

A B or T lymphocytic malignancy results in the clonal proliferation of a cell that therefore can carry a unique molecular fingerprint. Recombinant DNA technology now allows the recognition of this fingerprint when it is present in 0.1% to 1% of a total cell population. Therefore, molecular biology can increase the sensitivity of tumor detection in a tissue sample. It can classify tumors as being of T or B cell lineage, identify biclonal tumor populations, distinguish new primary lymphomas from relapsed cases, and address basic questions of tumor development and progression. This basic molecular technology can now be successfully combined with fine needle aspiration of lymph nodes to aid in the diag-

nosis and staging of patients with lymphomas. It may very likely become a routine test in the general work-up of patients with lymphoma at presentation and at relapse. There is an essential need to incorporate the information that will be generated from these tests into prospective clinical trials. After these trials it can be anticipated that routine management of patients with lymphoma will be influenced by molecular data as well as the other currently available analyses.

This technology is not restricted to use in the leukemias and lymphomas. Although the normal functional rearrangements that these cells undergo provide a special feature for studying lymphoid malignancies the molecular biological techniques are of general use. It is possible that gene systems in other cell types will undergo similar DNA configuration-altering recombination events as recombination signals exist outside of the immunoglobulin loci themselves, possibly in other gene systems. Of more likely usefulness, however, will be the ability to monitor DNA rearrangements and amplifications that are directly related to the development of a cancer and not just markers of the transformed cell type. This is a likely possibility as more and more specific cancers are being shown to be associated with distinctive chromosomal aberrations: translocations, amplifications, deletions, or inversions. On the level of the DNA, these aberrations often result in the development of distinguishing new configurations of DNA (a tumor-specific molecular fingerprint) often related to oncogenes; i.e. genes that are thought capable of contributing to the development of a malignant phenotype in a normal cell. Thus, a wide variety of tumors are approachable using the same basic technology. In many cases these chromosomal aberrations contribute to the etiology of cancers by altering the quality of quantity of RNA transcribed and translated from those genes structurally flanking the chromosomal aberrations. It is now possible to identify (at the level of individual cells within histologic tissue sections) those cells that are expressing a particular gene or genes. This technique is called RNA-RNA tissue in situ hybridization. Thus, technically, the potential exists to redefine histopathologic diagnoses on the basis of particular gene expression within tumor cells.

Recently a new technique, "polymerase chain reaction" or "PCR", has been developed that in certain conditions allows the identification of genomic rearrangements present in tumor samples at much less than 0.1% and also greatly increases the ability to identify particular mRNAs (or their corresponding cDNAs) of interest.

Over the next 5 years pathologists may begin reporting information about rearrangements, amplifications, and aberrant expression of various oncogenes in tumor biopsy specimens which an oncologist will then use to define prognosis or design a regimen of therapy. For the practicing oncologist, the molecular causes and consequences of a patient's disease will become relevant to determining that patient's diagnosis, course, and treatment.

#### Major Findings:

A spectrum of our work in progress and previous results.

Patient Studies:

The ability to detect immunoglobulin (Ig) and T cell antigen receptor (TCR) gene rearrangements is proving useful in confirming diagnosis of suspected B or T cell lymphoid malignancies and in establishing mono, oligo, and polyclonality in these and possibly related disorders.

Mycosis Fungoides, Sezary Syndrome, T-Gamma Lymphocytosis

In an early study we employed cloned probes for the TCR loci and Southern blot analysis (see references to basic molecular biological techniques at the end of reference list) to determine whether gene rearrangements were present in human T cell neoplasms representing various stages of T cell development. Gene rearrangements were present in all cases of immunophenotypic T cell disorders except a single case of T gamma lymphocytosis. Subsequent work by us and others have shown this disorder to be variable in terms of identification of clonal TCR rearrangements possibly reflecting disease heterogeneity and/or temporal evolution of clonality during the disease process. Germline gene configurations were present in all patients with "mature" non T cell neoplasms and in uninvolved tissue from patients with T cell lymphoid.

B Cell Precursor ALL of Childhood

An exhaustive study of over 80 patients seen at the NIH and within the Children's Cancer Study Group (CCSG) affiliated hospitals has been completed. The DNA was analyzed with Ig heavy, kappa, and lambda probes and TCR alpha, beta, gamma, and delta. There was marked genotypic heterogeneity in this population despite its immunophenotypic homogeneity. There was a possible correlation between germline genotype for all these loci and poorer course and prognosis. More definitively, we were able to demonstrate that the predominant genotypic pattern of immunophenotypic B cell precursor ALL of childhood was not the same as that observed in more "mature" B cell malignancies or in EBV transformed B lymphoblastoid lines. This raises questions about whether this ALL is truly a malignancy of a cell that is a normal precursor along the pathway of B cell differentiation.

Acute T Cell ALL of Childhood

TCR and Ig genes were examined in 26 cases of childhood T-ALL and 17 cases of pre B ALL. TCR gamma was rearranged in 22 of the 26, TCR beta in 23 of the 26, one or the other or both in almost every case of phenotypic T-ALL. Rearrangement of both alleles of the gamma and beta chains occurred in most T-ALLs. Expression of the beta and/or gamma chains was observed commonly in these tumors. Alpha chain gene expression was found less often, usually only in the most mature T-ALL's which were T3+. Analogous to studies of pre-B ALL molecular analysis of T-ALL suggests a hierarchy of early gamma and beta gene rearrangement followed by alpha, and a coordinate sequence of early appearance of the 3A1 antigen, followed by T11 and later T3. Three patients in this series with T-ALL also showed evidence of Ig heavy chain gene rearrangement. Of the 17 patients with pre-B ALL eight showed rearrangements of the TCR gamma gene. Evidence of activity of these two loci, felt to encode distinct differentiated functions, is much more commonly seen in these tumors of lymphocytes of less mature stages of development than in the more mature adult tumors such as those mentioned earlier.

### Acute ALL of Infancy

ALL in infants less than one year of age runs a particularly virulent course. It, therefore, was of interest to genotypically analyze a group of these patients to possibly contrast their patterns of gene rearrangement with those seen in older children as described above, as well as those seen in adults. Lymphoblasts of 11 infants demonstrated surface antigens which have been correlated with a pre-B cell phenotype. 4/11 of these retained the germline configuration of both Ig and TCR genes, suggesting that ALL in infancy represents an earlier stage of B cell development than is found in pre-B ALL of older children, where all had rearranged at least Ig H-chain genes. No phenotypic T-ALL patients were found in this study. As mentioned above, Ig H-chain rearrangements were occasionally seen in the series of T-ALL's, and TCR gene rearrangements, especially gamma, were identified in a large proportion (45%) of pre-B ALL's of older children. In contrast, TCR gamma gene rearrangements were not identified in pre-B ALL's of infants. These studies overlap studies of pre-B ALL in other children. Together they suggest discrete stages in lymphoid development vulnerable to malignant transformation. Because the distribution of ALL subtypes throughout childhood is not uniform, these data suggest either a change in size of the target cell population, or a differential vulnerability of lymphocyte subpopulations to etiologic agents with increasing age.

### Family Studies

Insertions, deletions, amplifications, and point mutations of genes occur in DNA throughout the evolutionary process. When these events occur in germline DNA, they are transmitted vertically from one generation to the next. Differing mutation sites and mutation rates between species and within a given species can be identified by comparison of defined populations by restriction enzyme site patterns for given probes of interest. This study of "restriction fragment length polymorphisms" ("RFLPs") reflecting the structural variability of DNA can be utilized in evolutionary, population, and family studies. Unlike the rearrangements of Ig or TCR genes, these RFLPs need not indicate the programmed rearrangement of a particular locus during the ontogeny of a particular cell type. Molecular genetic technology of DNA analysis can still be applied to this study. In what follows we describe one such example of RFLP analysis in which we have been involved. Its significance to the occurrence of a particular tumor in a particular family remains to be seen. Conceivably it could provide a marker for disease risk in this case. It would also be suggesting a mechanism of tumor formation akin to those now postulated, for example, for Wilm's tumor or retinoblastoma. We investigated a family in which a father and three of his offspring had meningioma with clinical onset at the ages of 35 to 65 years. A fourth offspring died of multiple neoplasms arising at 29 years. No one in the family had any evidence of von Recklinghausen disease. The three siblings with meningioma carried a constitutional Robertsonian translocation, t(14;22) (14qter cen 22qter), in peripheral blood leukocytes. Three other members of the second generation who were beyond the age at which the onset of meningioma is expected had no tumors and had normal karyotypes. In the third generation, whose members are now reaching the age for tumor onset, four carriers of the translocation have been identified; to date they are all asymptomatic except for one woman, who has breast cancer.

Both living members with meningiomas had a polymorphic variant of the c-sis oncogene in peripheral-leukocyte DNA, according to analysis with the Southern blot technique. This variation was also present in one asymptomatic member of the third generation and segregates with the morphologically normal No. 22 chromosome in both the affected and nonaffected members. It is possible that an "active" or mutant gene on the long arm of chromosome 22, possibly even the c-sis oncogene itself may be associated with the development of meningiomas in this kindred. Analysis of the meningioma tumor tissue itself would be of great interest in this regard but is presently not available. Sporadic meningioma is known to often to be associated with monosomy of chromosome 22 or less frequently the absence of the long arm of chromosome 22. The c-sis proto-oncogene has been localized to the tip of the long arm of chromosome 22. Studies are proceeding in collaboration with Dr. L. Ratner at Washington University, St. Louis to determine what, if any, role this c-sis polymorphism plays in the development of meningioma.

### Epidemiological Studies

We carried out a pilot feasibility study in collaboration with Dr. Ian Magrath of the Pediatric Branch, National Cancer Institute, USA and Dr. Gregory O'Connor, then Senior Scientist, International Agency for Research on Cancer, Lyon, France, to determine whether the logistics of tissue collection from widely dispersed and varied patient care centers could be set up so as to allow for appropriate and reproducible analysis of DNA rearrangements.

The most basic questions remain unanswered concerning worldwide presentation and distribution of different types of lymphoid leukemia and lymphoma. The initial screening assay on the patient samples was a determination of TCR beta and Ig heavy chain gene rearrangements. Samples were collected from Nagoya and Kyoto, Japan, from Riyadh, Saudi Arabia, from Lima, Peru, from two centers in Budapest, Hungary, and from New Delhi, India and sent to us via Lyon, France. We were easily able to study rearrangements of Ig and TCR loci in most of the samples received. Thus, it is reasonable to consider such international molecular genetic epidemiological studies. For a selected number of samples we extended our analysis to indicate studies of kappa and lambda light chain gene rearrangement and rearrangement of a select number of proto-oncogenes.

The ultimate goal of such an epidemiologic study would be to correlate the specific type of lymphoma with geographical distribution. In its most fundamental form this would involve relating the frequency of a specific Ig or TCR gene rearrangement and state of maturity of the tumor cell to the distribution of these malignancies in different parts of the world. Ultimately, the survey would be expanded to include information on oncogene expression and association with specific viruses. One need only consider how the recognition of endemic Burkitt's lymphoma in equatorial Africa or HTLV-1 positive acute T cell leukemia in the southern provinces of Japan or the Caribbean has been of such crucial importance for basic research as well as patient diagnosis and treatment. Often on the basis of DNA analysis alone one is able to make significant progress toward patient diagnosis and classification.



On a sample of lymph node from Riyadh, Saudi Arabia for which no clinical or laboratory data were available, we were able to discern a rearranged Ig heavy chain gene and kappa light chain gene, germline Ig lambda, TCR beta, and c-myc genes and a rearranged bcl-1 gene. On the basis of this analysis alone, it would be highly likely that such a patient suffered from a B cell follicular lymphoma with a t(14;18) translocation.

#### RNA-RNA Tissue In Situ Hybridization

As described in the previous section (as well as in the work of dozens of laboratories all over the world) multiparameter studies including cytochemistry, ultrastructural analysis, immunophenotyping, and recombinant DNA analyses of nucleic acids have contributed greatly in recent years to the classification and subclassification of lymphoid malignancies and other tumors. The kinds of recombinant DNA analyses described above examine the entire cell population, and thus the data represent an average of gene rearrangement or expression in which heterogeneity in the tumor can only be inferred. Recently it has become possible to use cloned nucleic acid probes at the level of individual cells in tissue sections. In conjunction with routine histologic diagnosis, this technique holds the promise of directly assessing cellular heterogeneity in a tumor, classifying tumor by their unique patterns of gene expression, and potentially gaining insight into etiologic and developmental questions in the disease state being analyzed.

As an early step in our utilization of this technique, we wished to establish the credibility of the data we would generate with it, with reference to the conventional techniques of molecular genetic analysis with which we were familiar and comfortable. We, therefore, conducted an analysis of an involved lymph node and peripheral blood from a patient with lymphadenopathy and a elevated white count. DNA and RNA from these tissues were analyzed by Southern and Northern blot, respectively, using <sup>32</sup>P probes derived from the Ig heavy and light and TCR beta chain loci. <sup>35</sup>S complementary RNA probes from these same loci were applied to fixed sections of these tissues and (after appropriate hybridization and washing conditions) autoradiographed.

We found the different techniques to be confirmatory and complementary. The technique is also consistent with immunophenotypic data generated on the same tissue. Our studies demonstrate that this patient suffered from a B cell malignancy. His lymph node showed a monoclonal B cell population and a polyclonal T-cell infiltrate. In his peripheral blood only the monoclonal B cell population was seen. Furthermore, in the malignant B cell population a provocative cellular heterogeneity was noted, a heterogeneity that showed an interesting distribution pattern within the lymph node studied. There was a diffuse clonal infiltrate of B lymphocytes expressing Ig mu and lambda message. Clustered around the vessels, however, were encountered apparently the same clonal population but with a much increased expression of the Ig mRNAs. The data and observations derived from this study supported our expectations that, when combined with clinical observations and other methods in histopathology, this kind of analysis could provide a general tool for answering important diagnostic and prognostic questions.

With this in mind we applied this technique to a study of myc related (c-myc, N-myc, L-myc) proto-oncogene expression in small cell lung cancer. The tissues investigated included cytopins of ten cell lines derived from patients with SCLC, four corresponding nude mouse xenografts from cell lines, and metastatic tumor tissue obtained by surgical biopsy and at autopsy. The expression of each gene was specifically demonstrated by autoradiography in the cytoplasm of the neoplastic cell samples. The average levels of oncogene expression in each specimen corroborated previous data obtained by Northern blot assay. In addition, heterogeneity in gene expression from cell to cell in each sample was noted. This study represented the first attempt to demonstrate oncogene expression in lung cancer cells in situ, and confirmed that the expression of these myc related genes can be seen in the primary tumor.

We have used tissue in situ hybridization in conjunction with direct sequencing of RNA to demonstrate the utility of immunoglobulin and T cell receptor gene rearrangements as lymphoid tumor specific markers. We obtained the sequence of a CDR3 segment expressed by a malignant lymphocyte clone, made an oligonucleotide complementary to it, and used this complementary oligonucleotide to identify malignant cells in tissue sections. This protocol (or a variation of it and utilizing polymerase chain reaction to amplify and obtain sequence or genes expressed at less than 0.1% of total message) will be of potential use in the diagnosis and staging of lymphoid malignancies as well as in the determination of minimal residual diseases.

### Chromosome In Situ Hybridization and Gene Mapping

Our group was one of those early involved in the localization of genes in the human genome by the technique of chromosome in situ hybridization following the technique first developed by Harper and Saunders. Since our first effort localizing the Ig heavy chain locus to 14q32, we have conducted numerous other studies. These studies have included the localization of c-myc to 8q24, the localization of the beta globin cluster to 11p15 (in collaboration with Dr. Cynthia Morton) the localization of L-myc to 1p32 and of an amplified c-myc to an HSR in a small cell lung cancer cell line (in collaboration with Dr. John Minna and Dr. June Biedler). We have localized the human beta 1-4 galactosyltransferase to 9p13 (within the same chromosome band as is found in the gene defective in the disease galactosemia). In collaboration with Dr. Tak Mak we localized the gene for the TCR alpha chain locus to 14q11.2. In collaboration with Dr. James Battey we localized the gene for human gastrin releasing peptide to 18q21. This was the same site to which we, in collaboration with Dr. Stan Korsmeyer and others, had localized the bcl-2 gene. We have also utilized this technique as part of our molecular genetic analyses of chromosomal aberrations including the human T cell lymphoma line SUP-T1, a human myeloma cell line H929, two patient samples carrying a t(14;14), one patient with a t(1;14) in his leukemic cells, one with a t(X;14) lymphoproliferation, and one with Ewing's sarcoma and a t(11;14;22) complex translocation. We are now mastering the technique of chromosome in situ hybridization using biotinylated probes. Recently this method has increased the sensitivity and specificity of this procedure allowing for more rapid gene mapping and even the mapping of genes or transcripts in interphase nuclei.

Tumor Genotyping Service

The establishment of a service facility for processing of tissue samples, and DNA and RNA preparation. The capacity to comprehensively screen selected patient populations or tumor samples for the rearrangement and/or expression of particular genes of interest. We are interested in maintaining a supervisory and quality control role over the activities of this laboratory. Envisioned is a NIH wide facility with a board composed of members from different institutes meeting to decide what questions should be addressed and to review the data being generated.

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<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 06589-05 NMOB
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biology and in vitro Growth and Drug Sensitivity Testing of Lung and Other Cancers		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Adi F. Gazdar, MD	Senior Investigator NCI-NMOB
Others:	Ilna Linnoila, MD	Senior Investigator NCI-NMOB
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	John Minna, MD	Chief NCI-NMOB
COOPERATING UNITS (if any) Pediatric Oncology Branch, Medicine Branch, Clinical Pharmacology Branch, Radiation Oncology Branch, Laboratory of Molecular Biology		
LAB/BRANCH NCI-Navy Medical Oncology Branch		
SECTION Human Tumor Biology		
INSTITUTE AND LOCATION NCI, DCT, COP, Naval Hospital, Bethesda, MD		
TOTAL MAN-YEARS: 7	PROFESSIONAL: 5	OTHER: 2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A. There appears to be an increase in the incidence of adenocarcinoma subtype of NSCLC in the USA. In particular, tumors with features characteristic of bronchiolo-alveolar carcinomas appear to be increasing. The relatively large number of cell lines that we have established that have ultrastructural and biochemical evidence of arising from peripheral airway cells (Clara or Type II pneumocyte) confirms these findings.</p> <p>B. A randomized prospective clinical study of extensive disease SCLC demonstrates that in vitro chemosensitivity testing accurately predicts the clinical response of the patients to initial as well as to secondary therapy. In addition, there may be a modest benefit to administering individualized drug assay based therapy. Our studies indicate that patients predicted to fail conventional initial or individualized secondary therapy may be offered alternative forms of therapy. In a separate analysis, the clinical responses of extensive stage lung cancer patients to initial therapy was highly correlated with the results of the IC<sub>50</sub> values obtained by the MTT assay.</p> <p>C. Panels of cell lines of SCLC, NSCLC, colorectal and gastric carcinomas are useful reagents to screen putative new phase I and II drugs using the MTT tetrazolium dye assay.</p> <p>D. Transfection of the c-myc and H-ras proto-oncogenes into a non-transformed rat cell line resulted in increased cellular resistance to several cytotoxic agents. Thus, amplification/over-expression of oncogenes may be one mechanism by which tumor cells become drug resistant.</p>		

## PROJECT DESCRIPTION

Biology and in vitro Growth and Drug Sensitivity Testing of  
Lung and Other Cancers

PI:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
Others:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
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	John Minna, MD	Chief	NCI-NMOB

Collaborating Branches:

Pediatric Oncology Branch, Medicine Branch, Clinical Pharmacology Branch,  
Radiation Oncology Branch, Laboratory of Molecular Biology

Objectives:

One of the major objectives of this Branch is to develop newer, more rational therapies for the cancer types representing our major clinical interests. By studying the biology of specific cancers in depth, new ideas for cancer control are generated, tested in vitro and brought to clinical trial. We presume that such an approach is more likely to advance in the therapy of refractory tumors such as colon and non-small cell lung cancer (NSCLC) than the development of new non-targeted cytotoxic agents. Further, a comprehensive knowledge of the biology of a cancer type can aid the physician in interpreting certain clinical phenomena such as hormone secretion, tumor progression, etc. Finally, identification of tumor markers may aid diagnosis, staging, detection of relapse, imaging, sub typing, and monitoring response to therapy.

Three of the currently active clinical protocols for the therapy of SCLC and NSCLC depend on the selection of individualized patients' chemotherapy by in vitro drug sensitivity testing. Thus one of the major objectives of the Branch is to develop methods to: 1) amplify tumor cells so that adequate numbers are available for testing; 2) develop and apply rapid, accurate, reproducible testing procedures; 3) demonstrate the clinical relevance of the testing procedures; and 4) utilize in vitro testing for biological and preclinical studies.

Major Findings:The Changing Histopathology of Lung Cancer

We have noted previously an increased incidence of adenocarcinoma in our non-SCLC (NSCLC) protocol patients (63% of all NSCLC). We also noted that peripheral adenocarcinomas with some or all of the features of bronchioloalveolar (BAC) carcinomas appeared to be common (about 50% of all adenocarcinomas, or about 30% of all NSCLC tumors). We have extended these findings by reviewing the pathology of resected lung cancer at Johns Hopkins Hospital, Baltimore. We

are currently analyzing cases from five institutions on three continents (Asia, Europe, N. America) to determine the incidence of NSCLC subtypes worldwide.

### In Vitro Drug Sensitivity Testing Clinical Correlation

The Weisenthal dye exclusion assay is used to test clinical specimens from patients entered onto therapeutic trials for lung cancer. The largest and best studied base currently available is from the Extensive Stage small cell lung cancer protocol, #83 13 (also see report by Dr. D. Ihde). Samples were obtained from 79/80 patients entered onto protocol (99%), and at least one tumor containing sample was obtained from 60 patients (75%). Cell lines were established from 28 patients from whom at least one tumor containing specimen was obtained (33% of all patients). Several parameters of in vitro drug sensitivity were significantly associated ( $p_2 < 0.05$ ) with clinical response to primary therapy and also with response to the "in vitro best regimen", and were marginally associated with length of survival ( $0.07 \leq p_2 \leq 0.08$ ). Sixteen patients (23%) received their "in vitro best regimen" as secondary therapy, and four of these (25%) attained a complete response, compared to 3/43 (7%) receiving an empiric regimen ( $p_2=0.16$ ). Our conclusions from this study are as follows: 1) selection of individualized chemotherapy is labor intensive but feasible in extensive stage small cell lung cancer; 2) drug sensitivity testing data are associated with clinical response to primary therapy and to secondary therapy with an "in vitro best regimen"; and 3) individualized chemotherapy may be of modest therapeutic efficacy.

In a separate analysis, we compared the responses of patients with extensive stage lung cancer, both SCLC and NSCLC, to initial therapy with etoposide/cis-platin with the in vitro sensitivities of cell lines established from their tumors, using the MTT assay. Patients were restaged after 12 weeks of therapy and classified as responders or non-responders. There was a highly significant correlation between IC<sub>50</sub> values of both drugs and clinical response.

### DST Studies - Non Clinical

We have previously described the use of MTT tetrazolium dye assay for the extensive study of the in vitro sensitivity patterns of lung and colorectal carcinomas. These studies validate the concept that cell lines are suitable models for pre clinical studies. We have extended these studies to gastric carcinoma. We performed a comparison of the in vitro sensitivity patterns to cytotoxic drugs and expression of the multidrug resistance associated MDR1 gene in four gastric carcinoma cell lines and compare the results to those of a panel of 11 colorectal carcinoma cell lines. In addition, we tested the effects of leucovorin on enhancement of fluorinated pyrimidine-induced cytotoxicity.

Our studies demonstrated several interesting differences between gastric and colorectal carcinoma cell lines. Using the MTT assay, the gastric lines were more sensitive to some drugs, including doxorubicin and cisplatin, but not to the fluorinated pyrimidines. Addition of leucovorin at a clinically achievable concentration enhanced the cytotoxic effects of both 5-fluorouracil and 5-fluoro-2'-deoxyuridine (FUdR) in colorectal lines, but only enhanced the effects of FUdR in gastric lines. Using a slot blot assay, in general, our findings

reflect clinical experience, and may help in the design of clinical studies in gastrointestinal malignancies.

Because serum inhibits the in vitro cytotoxicities of certain antineoplastic agents, we investigated the inter-relationships between medium type, cell proliferation and cytotoxic effect. Twenty four human lung cancer cell lines were tested with nine anticancer agents in both medium types. To determine the influence of culture media on cytotoxicity, we analyzed the data only from lines that replicated equally efficiently in both media. Serum supplemented media had a negative effect on the cytotoxic action of some drugs (especially methotrexate, 5-fluorouracil and, to a lesser extent, mitomycin-C) independent of its effects on cell proliferation. Our results demonstrate that fully defined SFM are suitable for use in in vitro cytotoxic assays for selection of individualized therapy or for screening of new neoplastic agents, and may increase the number of antineoplastic agents that can be tested satisfactorily.

#### The Role of the MDRI Gene in Human Cancers

Multi-drug resistance plays a major role in the response of lung cancers to chemotherapy. Last year we described the results of a comprehensive study of the role of the human MDRI gene in lung cancer. These studies may be summarized as follows: low levels of MDRI RNA are present in most types of lung cancer and normal lung; and expression is not correlated with prior therapy status or with the results of in vitro drug testing. Thus, the MDRI gene plays little or no role in lung cancer, and the major mechanism of drug resistance must be due to other factor(s).

We have extended these studies to GI malignancies. In contrast to lung cancer, colorectal carcinoma tumors and cell lines expressed relatively high levels of mRNA. Levels in gastric lines were lower than in colorectal lines, reflecting the lower expression in normal gastric mucosa and the high levels in intestinal mucosa. This differential expression in gastric and colorectal lines may reflect the increased chemosensitivity profiles of the former (see above).

#### Establishment and Characterization of Gastric Carcinoma Cell Lines

We have established and characterized four continuous cell lines derived from human primary and metastatic gastric carcinomas, and have compared their properties with a panel of colorectal carcinoma cell lines previously established and reported by us. Our success rate in culturing gastric carcinomas was relatively low, especially from primary tumors, compared to colorectal carcinoma. These observations probably reflect the relatively modest number of gastric carcinoma cell lines established (mainly from Japan) compared to the abundance of colorectal carcinoma lines established worldwide. The multiple properties we have demonstrated in our gastric carcinoma cell lines are remarkably similar to those found in the panel of colorectal carcinoma cell lines. These properties include morphology, growth characteristics, expression of surface glycoproteins (CEA, CA 19-9, TAG-72), partial expression of NE cell markers, frequent chromosomal evidence of gene amplification, and occasional amplification of the c-myc proto-oncogene. Our four well characterized cell lines should provide useful additions to the modest number currently available for in vitro studies of gastric carcinoma.

### The Effects of Oncogene Transfection on in vitro Drug Sensitivity

Spontaneous amplification of the c-myc gene in human lung cancer cells and transfection of ras family oncogenes into NIH 3T3 cells are associated with resistance to ionizing radiation. We determined whether increased expression of myc or ras genes in rat cells results in increased resistance to cytotoxic drugs. The rat fibroblast cell line Rat-1a was transfected with the following mammalian expression constructs: 1) an activated H-ras gene; 2) a murine c-myc gene; and 3) the human L-myc gene. The clones so obtained expressed moderate to high levels of RNA of the appropriate oncogene (which were relatively low or undetectable in the parent line). The clones were tested for their in vitro sensitivity to 7 cytotoxic agents (etoposide, cisplatin, doxorubicin, nitrogen mustard, vinblastine, bleomycin and carmustine) using the semi-automated MTT assay. Controls included the parent line and a ras transfected clone lacking detectable levels of activated ras RNA. Each experiment (performed in replicates of 8) was repeated 3-8 times. Because of multiple comparisons, a test was considered significant at the 5% level only if the p value of the Mann-Whitney test was <0.007. The clones were significantly more resistant (1.4 to 8 fold) than the parent line as follows: clone H-ras 1-1 for 5/7 drugs; clone c-myc 2-9 for 4/7 drugs; clone and L-myc 6-7-2 for 0/6 drugs. We conclude that productive transfection of the H-ras and c-myc, (but not L-myc) genes may result in increased resistance to multiple drugs.

### Future Studies:

We are performing an analysis of the typing of NSCLC worldwide. Sites to be analyzed will include co-operating institutions in North America, Europe and Japan. These studies will be complemented by more limited correlations between histopathology and ultrastructure and immunohistochemistry. By these techniques we will be able to confirm our light microscopic observations regarding the increase in adenocarcinomas, and its BAC subtype. Finally, we will determine whether the observations we have made are due to differences in incidence or altered diagnostic techniques, or to both. We will study the distribution of NSCLC types at Johns Hopkins Hospital in the sixties compared to recent past years.

We will continue our current clinical protocols for SCLC and NSCLC based on in vitro selected therapy. Data will be correlated with the patients' responses. Preclinical studies will include testing of phase I and II drugs and correlating in vitro predictions with clinical results. As amplification and overexpression of the myc gene family are relatively common in lung cancer, especially previously treated SCLC, we will continue to study the relationship between oncogene expression and in vitro chemosensitivity and radioresistance. We will test both lung cancer cell lines as well as rat cell lines transfected with various oncogenes.

Disease orientated panels of cell lines will be used to test potential and actual phase I and phase II agents, both for correlation of in vitro results with clinical response and for the selection of agents to test in future phase I trials in NSCLC.



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<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 CM 06594-04 NMOB
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Genetic Events in Lung Cancer		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Bruce E. Johnson, MD	Senior Investigator NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator NCI-NMOB
	John D. Minna, MD	Chief NCI-NMOB
	Daniel C. Ihde, MD	Prof Med NCI-USUHS NCI-NMOB
	James Mulshine, MD	Senior Investigator NCI-NMOB
	John Brennan, MD	Instructor Med NCI-USUHS NCI-NMOB
	David Bliss, MD	Biotechnology Fellow NCI-NMOB
COOPERATING UNITS (if any) Jaqueline Peng, MD, Medicine Branch, COP, DCT, NCI; Bert Zbar, MD, Immunobiology Branch, BRMP, DCT, NCI; Susan Naylor, PhD, Department of Cellular and Structural Biology, University of Texas		
LAB/BRANCH NCI-Navy Medical Oncology Branch		
SECTION Human Tumor Biology and Molecular Genetics and Immunology		
INSTITUTE AND LOCATION NCI, DCT, COP, Naval Hospital, Bethesda, MD		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	2.4	0.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>A. Restriction fragment length polymorphism (RFLP) studies have shown that 33 of 35 patients with small cell lung cancer (SCLC) lose heterozygosity in the tumor tissue DNA that was present in normal tissue DNA. This confirms the loss of genetic information localized to the short arm of chromosome 3 previously described by karyotype analysis. Extrapulmonary small cell cancers have been studied using karyotype and RFLP analysis and 4/5 patients retain both chromosome 3's in the region of 3p14-21 by both RFLP analysis and karyotype analysis. This demonstrates a different genetic lesion in tumors that histologically are indistinguishable.</p> <p>B. The tumors and tumors cell lines from patients with small cell lung cancer and the syndrome of inappropriate antidiuretic hormone (SIADH) were studied for production of arginine vasopressin (AVP) mRNA production. Two of 5 patients with SIADH made AVP mRNA by Northern blot analysis and S1 nuclease analysis. Three of the 5 make atrial natriuretic factor (ANF) by Northern blot analysis and S1 nuclease analysis. Radioimmunoassay confirmed the presence of immunoreactive ANF and AVP in the tumor cell lines. This is the first report of ectopic ANF production in SCLC.</p>		

## PROJECT DESCRIPTION

## Molecular Genetic Events in Lung Cancer

Professional Staff:

PI:	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
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	James Mulshine, MD	Senior Investigator	NCI-NMOB
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	David Bliss, MD	Biotechnology Fellow	NCI-NMOB

Collaborating Branches:

Jaqueline Peng, MD, Medicine Branch, COP, DCT, NCI; Bert Zbar, MD, Immunobiology Branch, BRMP, DCT, NCI; Susan Naylor, PhD, Department of Cellular and Structural Biology, University of Texas

Objectives:

1. Simultaneously study normal tissue, tumor cell lines, and tumors from the patients for chromosomal deletions using restriction fragment length polymorphisms while having a karyotype performed on the cell line to correlate the evidence for chromosomal deletion using restriction fragment polymorphisms and cytogenetic analysis.
2. Study tumor cell lines and tumors from patients with SCLC and hyponatremia for evidence of ectopic AVP or ANF production and correlate this with the patients fluid and electrolyte status.

Major Findings:

Restriction Fragment Polymorphism Studies Show Consistent Deletion of Loci on the Short Arm of Chromosome 3 in Small Cell Lung Cancer Patients' Tumors Compared to Their Normal Tissue

In collaboration with Dr. Sue Naylor and Dr. Berton Zbar, we have prepared DNA from normal and tumor tissue or tumor cell line from the same patient and analyzed the tumor DNA for loss of heterozygous loci on chromosome 3p in the tumor tissue compared to normal tissue from the same patient. They have evaluated the normal tissue DNA with DNA fragments that detect heterozygous loci on chromosome 3p, D3S2, D3S3, and DNF15S2. The normal tissue had heterozygous loci within chromosome 3p14-23 in 35 of the 37 DNAs analyzed. The tumor was reduced to homozygosity in one of these loci in 33 of the 35 patients (94%). Thus, by using this restriction fragment polymorphism analysis, the tumor DNA shows loss genetic information from at least a portion of the short arm of one chromosome 3 in 33 of the 37 patients. This lends support to the previous karyotypic description of a consistent deletion of the short arm of chromosome 3 in small cell lung cancer.

The Short Arm of Chromosome 3 is not Lost in Extrapulmonary Small Cell Cancer

One of the patients who did not lose heterozygosity in this tumor tissue DNA compared to the normal tissue DNA was a patient who had extrapulmonary small cell cancer arising in his prostate gland. Therefore, we identified 5 extra pulmonary small cell cancer patients who had tumor cell lines or tumor plus normal tissue available for study. These 5 patients had their tumors arise in the prostate gland, cervical lymph nodes, brain, uterus, and cervix. We then studied the patients' 4 tumor cell lines with karyotype analysis. The 4 tumor cell lines, 3 tumors and 3 normal tissue DNAs were studied with three probes which detect restriction fragment length polymorphisms within 3p14-21. The karyotype analysis showed all 4 tumor cell lines retain at least 2 chromosome 3's in the 3p14-21 region. The restriction fragment length polymorphism studies demonstrated the presence of heterozygous alleles in the 3p14-21 region with one exception. The patient who presented with small cell cancer in their cervical lymph nodes showed a reduction to homozygosity in this tumor DNA at the D3S2 locus (3p14-21). We believe it is interesting that this was the only patient who presented with metastases to the lymph nodes and did not present with an identifiable primary small cell cancer like the other 4 patients studied.

These studies of extrapulmonary small cell cancer have shown that in contrast to the consistent deletion of the 3p14-23 region in small cell lung cancer, extra pulmonary small cell cancer typically retains both arms of chromosome 3.

Tumors and Tumor Cell Lines from Patients with Small Cell Lung Cancer and SIADH Produce Ectopic Atrial Natriuretic Factor

Hyponatremia in patients with small cell lung cancer can be caused by tumor production of arginine vasopressin (AVP) and resulting in the syndrome of inappropriate antidiuretic hormone (SIADH). However, AVP peptide has not always been present in analyzed tumor specimens. We therefore examined tumors and tumor cell lines from 5 patients with small cell lung cancer and SIADH for AVP mRNA. The specimens from 2 of the 5 patients expressed AVP mRNA. RNA samples from the 3 patients with undetectable AVP mRNA expressed abundant atrial natriuretic factor (ANF) mRNA. AVP and ANF peptide levels in tumor cell line lysate preparations from four of these patients were tested by radioimmunoassay and confirmed the gene expression data. These studies demonstrate ectopic production of ANF mRNA in small cell lung cancer from patients with this cancer and SIADH. Atrial natriuretic factor may be a candidate peptide for causing sodium abnormalities in patients with small cell lung cancer.

Publications:

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3. Johnson BE, Simmons A. myc family DNA amplification in small cell lung cancer cell lines and tumors. Lung Cancer 1988;4:131-134.
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<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 06595-03 NMOB
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinically Relevant Immunohistochemical Markers in Lung Cancer		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Ilona Linnoila, MD Others: James Mulshine, MD Adi Gazdar, MD	Senior Investigator Senior Investigator Senior Investigator	NCI-NMOB NCI-NMOB NCI-NMOB
COOPERATING UNITS (if any) Biostatistics and Data Management Section, Clinical Oncology Program, DCT, NCI, (Seth Steinberg, PhD), Anatomic Pathology, Naval Hospital and Anatomic Pathology, NCI, NIH		
LAB/BRANCH NCI-Navy Medical Oncology Branch		
SECTION Human Tumor Biology		
INSTITUTE AND LOCATION NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814		
TOTAL MAN-YEARS: 4	PROFESSIONAL 3	OTHER 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Our goal is to define immunohistochemical markers that will best type lung cancer for diagnosis, prognosis, and selection of therapy. Small cell lung cancer (SCLC), characterized by neuroendocrine (NE) features, is responsive to chemo- and radiotherapy. Some non-SCLC also express NE features. The hypothesis is that these tumors might be more responsive to cytotoxic treatment than other non-SCLC.</p> <p>A. <u>Characterization of markers.</u> In a retrospective study a comprehensive group of 113 lung cancers were tested for the immunohistochemical expression of 17 antigens using a sensitive avidin-biotin-peroxidase technique. Logistic regression analysis was used to separate tumors into the proper categories (SCLC and carcinoid tumors versus NSCLC) based on the immunohistochemical markers. As a result 95% of the tumors were correctly predicted using the cell counts and staining intensities of only six markers. The results suggested that 1) individual marker counts are not useful in tumor classification, 2) "specific" NE markers such as serotonin and neuropeptides bombesin, calcitonin, ACTH, vasopressin, neurotensin are not useful, 3) the best NE markers are a panel of "general" NE markers (Chromogranin A, Leu 7, NSE) which are present in NE cells throughout the body.</p> <p>B. <u>Clinicopathologic correlation.</u> This panel of "general" NE markers was applied to the non-SCLC cases on protocol 83-15 in our branch. Although the numbers were small, the response rate to chemotherapy was 50% (4/8) in the patients whose tumors were positive for NE markers versus 16% (6/38) in those with negative NE markers. Moreover, patients with NE positive tumors developed metastases significantly earlier (<math>p2 &lt; 0.027</math>). The significance of the project lies in the possible identification of prognostically important clinical subsets of lung cancer. Immunohistochemistry provides a highly effective and specific technique to achieve this goal.</p>		

## PROJECT DESCRIPTION

## Clinically Relevant Immunohistochemical Markers in Lung Cancer

Professional Staff:

PI:	Iлона Linnoila, MD	Senior Investigator	NCI-NMOB
Others:	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Adi Gazdar, MD	Senior Investigator	NCI-NMOB

Collaborating Branches:

Biostatistics and Data Management Section, Clinical Oncology Program, DCT, NCI, (Seth Steinberg, PhD), Anatomic Pathology, Naval Hospital and Anatomic Pathology, NCI, NIH

Objectives:

There are four major histological types of lung cancer, namely small cell lung cancer (SCLC) (25%), adenocarcinoma (25%), squamous cell carcinoma (30%) and large cell carcinoma (15%). For a number of biological and clinical reasons, these lung carcinomas may be divided into SCLC and non-SCLC (NSCLC) tumors. SCLC and the rare bronchial carcinoid express many neuroendocrine (NE) features including dense core granules by electron microscopy, high levels of the key amine producing enzyme L-dopa decarboxylase, and the glycolytic isoenzyme neuron-specific enolase (NSE) and hormone or neuropeptide production.

SCLC unlike NSCLC is extremely sensitive for chemotherapy and radiation, and there are scattered reports on favorable responses to chemotherapy by "atypical endocrine" tumors of the lung. This knowledge together with the recently established NE markers has prompted us to explore if 1) the expression of neuroendocrine markers in NSCLC is associated with favorable response to chemo- or radiotherapy, and 2) if the degree of expression of neuroendocrine markers in SCLC correlates with clinical outcome. Immunohistochemical technique provides a readily applicable tool for this.

Methods Employed:

1. Tumors. A comprehensive group of 113 primary lung cancers was chosen from the archives of the departments of pathology at the Bethesda Naval Hospital and National Cancer Institute. In addition, tumor material was obtained also from the patients on NCI protocol 83-15. Serial sections from routinely processed paraffin blocks were used.

2. Antibodies. The application of immunologic techniques that use hormone markers has been hampered by the fact that tumors with similar histologic and cytologic features may produce a variety of immunoreactive substances, and some tumors may synthesize more than one hormone. Recently, a mouse monoclonal antibody LK2H10 produced against human pheochromocytoma has been shown to be directed against chromogranin A (ChrA) a constituent of secretory granules in most peptide producing endocrine cells. The demonstration of chromogranin in

lung tumors serves as a useful marker for a broad spectrum of lung tumors with NE features including SCLC and the rare bronchial carcinoid. Other general immunohistochemical markers for NE differentiation include monoclonal antibody Leu-7 (HNK-1). Leu-7 reactivity was originally identified in subpopulation of lymphocytes called natural killer cells and later noted to be present also in nerves and wide variety of endocrine cells. Antibodies to NSE also react with nerves and cells of the diffuse NE system and its tumors. The advantage of applying such general NE markers is that they provide a more uniform recognition for multiple NE tumors that may in turn synthesize a variety of specific products such as different hormones.

3. Immunohistochemical Staining. Staining was performed using the avidin-biotin peroxidase technique. Appropriate positive and negative controls were included in each assay. Results of the immunostaining were reviewed scoring both for the intensity of the staining and number of positive cells.

#### Major Findings:

1. Characterization of Markers. We were able to demonstrate that the majority of cells in most SCLC and all carcinoid tumors were positive for the general NE markers and many hormones. Logistic regression analysis was used to separate tumors into the proper categories on the basis of markers and 95% the tumors were correctly classified applying a model created from staining indexes of general NE markers (ChrA, Leu 7, NSE). Evaluation of the expression of multiple markers revealed that 7/77 NSCLC had a staining pattern indistinguishable from SCLC.

We have concluded that 1) Application of the general NE markers produces acceptable classification of lung tumors; 2) Most but not all SCLC and carcinoids express multiple NE markers in a high percentage of tumor cells; 3) Occasional NSCLC show staining patterns indistinguishable from SCLC; 4) Many NSCLC contain a small subpopulation of cells expressing NE markers.

2. Clinicopathologic Correlation. The panel of "general" NE markers (ChrA, Leu7, NSE) was applied to the non-SCLC cases on protocol 83-15 ("Treatment of Non-Small Cell lung Cancer Utilizing In Vitro Drug Sensitivity"). Based on a detailed histopathological evaluation of tumor specimens of the patients already entered in the protocol it appears that in over 80% of the cases such an immunohistochemical analysis on untreated patient specimens can be performed. Currently we have stained 101 of the 133 cases entered and in 20/98 (20%) non-SCLC at least two out of the three general NE markers were positive. The results of the first 80 cases are summarized in the following table as an example:

GENERAL NE MARKERS IN NSCLC BY HISTOLOGICAL TYPE  
(80 CASES ON PROTOCOL 83-15)

(% positive)	Chr A	Leu 7	NSE
Adenocarcinoma	2/45 (4)	10/45 (22)	22/45 (49)
Large cell	6/19 (32)	3/19 (16)	9/19 (47)
Epidermoid	0/11 (0)	2/11 (18)	4/11 (36)
Other	0/2 (0)	0/2 (0)	1/2 (50)
TOTAL NSCLC	8/77 (10)	15/77 (19)	35/77 (45)
Carcinoid	3/3 (100)	3/3 (100)	3/3 (100)

The updated analysis of the response rate to chemotherapy in those 122 non-SCLC patients on protocol 83-15 in correlation with the results of immunohistochemistry revealed a rate 50% (4/8) in the patients whose tumors were positive for at least 2 out of 3 general NE markers versus 16% (6/34) in those with negative NE markers. There was also a strong correlation of the expression of immunohistochemical NE markers with other biochemical markers for NE differentiation, such as L-dopa decarboxylase levels in tumors. While there was no difference in survival between patients whose tumors were NE positive and other non-SCLC, patients with NE positive tumors developed metastases significantly earlier ( $p < 0.027$ ).

Significance to Biomedical Research and the Program of the Institute:

The significance of the project lies in the possible identification of prognostically important clinical subsets of lung cancer. There are at least 150,000 new cases of lung cancer (75% of which are non-SCLC) discovered annually. Our preliminary results support our hypothesis that non-SCLC which express NE features might be more responsive to cytotoxic treatment than other non-SCLC. Immunohistochemistry provides a highly effective and specific manner to screen for these tumors.

An important, practical aspect of this study is that it will provide a valuable archive of large patient material with well characterized clinical data. This enables statistically meaningful correlations allowing systematic evaluation of the biological significance of defined markers.

Proposed Course:

1. The expression of markers will be correlated to the clinical data including performance status, best response, and survival. At the end of the protocol 83-15 we should have accumulated results on 120 patients, if 150 patients are accrued as planned. This will provide a basic correlation.

2. Based on our initial observations we expect that 10-20% of non-small cell lung cancers express neuroendocrine markers. In order to extend the analysis and reach meaningful clinical correlations we have initiated a collaboration with the ECOG (Eastern Cooperative Oncology Group) and LSCG (Lung Cancer Study Group). ECOG and LSCG have full clinical response and survival information on nearly 2,800 treated patients. A large number of these have pretreatment tumor

samples available for analysis. We plan to study the expression of general NE markers chromogranin, Leu 7 and NSE, as well as selected other markers, and relate this to tumor type, response to therapy, and survival. At the present time, we have stained 400 cases, and the interim analysis revealed that 12-16% of the cases were positive for at least two out of the three general NE markers, thus confirming our initial findings in independent tumor sets.

Our preliminary analysis of other common immunohistochemical tumor markers in the LCSG cases revealed that patients whose tumors lacked mucin, which is a secretory product of many adenocarcinomas, had an average 5 year recurrence free survival, while the most mucin positive cases had an average recurrence free survival of about one year. Also, patients with elevated tissue staining for carcinoembryonic antigen, CEA, in their tumors had a more favorable outcome.

#### Publications:

1. Cuttitta F, Fedorko J, Gu J, Lebacqz-Verheyden AM, Linnoila RI, Battey JF. Gastrin releasing peptide gene associated peptides (GGAPS) are expressed in normal human fetal lung and small cell lung cancer. A novel peptide family found in man. *J Clin Endocrinol Metab* 1988;67:576-582.
2. Gu J, Linnoila RI, Seibel, NL, Gazdar AF, Minna JD, Brooks BJ, Hollis GF, Kirsch IR. A study of myc related gene expression in small cell lung cancer by in situ hybridization. *Am J Pathol* 1988;132:13-17.
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6. Travis WD, Linnoila RI, Horowitz M, Pass H, Ozols R, Gazdar AF. Pulmonary nodules resembling bronchioloalveolar carcinoma in two adolescent cancer patients. *Modern Pathol* 1988;1:372-377.
7. Gazdar AF, Linnoila RI. The pathology of lung cancer: Changing concepts and newer diagnostic techniques. *Semin Oncol* 1988;15:215-225.
8. Rapp VR, Huleihel M, Pawson T, Linnoila I, Minna JD, Heidecker G, Cleveland JL, Beck T, Forchhammer J, Storm SM. Role of raf oncogenes in lung carcinogenesis. *Lung Cancer* 1988;4:162-167.

9. Ihde DC, Russell EK, Oie HK, Linnoila RI, Steinberg SM, Ghosh BC, Schumacher HR, Minna JD, and Gazdar AF. Prospective clinical trial of individualized chemotherapy based on *in vitro* drug sensitivity testing in extensive stage small cell lung cancer. In: Salmon SE, ed. *Adjuvant Therapy of Cancer V*. Orlando: Grune & Stratton 1987;201-205.
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16. Gazdar AF, Russell EK, Oie HK, Steinberg SM, Ghosh BC, Linnoila RI, Minna JD, Ihde DC. Extensive disease small cell lung cancer: A prospective trial of chemotherapy based on *in vitro* drug sensitivity testing. In Marangolo M, Fiorentini G, eds. *Small Cell Lung Cancer. Advances in the Biosciences*. Oxford: Pergamon Press 1988;173.
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20. Jensen SM, Russell EK, Gazdar AF, Linnoila RI. Synaptophysin in a sensitive and specific marker for neuroendocrine differentiation in lung cancer and extra-pulmonary small cell carcinoma cell lines. Cancer Res, in press.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 06596-03 NMOB																
PERIOD COVERED October 1, 1988 to September 30, 1989																		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) In Vitro Drug Testing for Limited SCLC and Phase I Drug Development																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Bruce E. Johnson, MD</td> <td style="width: 35%;">Senior Investigator</td> <td style="width: 15%;">NCI-NMOB</td> </tr> <tr> <td>Others:</td> <td>Daniel C. Ihde, MD</td> <td>Prof Med NCI-USUHS</td> <td>NCI-NMOB</td> </tr> <tr> <td></td> <td>Adi F. Gazdar, MD</td> <td>Senior Investigator</td> <td>NCI-NMOB</td> </tr> <tr> <td></td> <td>John D. Minna, MD</td> <td>Chief</td> <td>NCI-NMOB</td> </tr> </table>			PI:	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB	Others:	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB		Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB		John D. Minna, MD	Chief	NCI-NMOB
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COOPERATING UNITS (if any) Eli Glatstein, MD, Richard Deming Radiation Oncology Branch; John Strong, PhD, Robert Parker, PhD, Biological Chemistry Branch and Hoo Geung Chun, MD, Investigational Drug Branch																		
LAB/BRANCH NCI-Navy Medical Oncology Branch																		
SECTION Human Tumor Cell Biology and Molecular Genetics and Immunology																		
INSTITUTE AND LOCATION NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814																		
TOTAL MAN-YEARS: 2.1	PROFESSIONAL: 1.5	OTHER: 0.6																
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>A protocol combining twice a day radiotherapy plus VP 16 and cisplatin for limited stage small cell lung cancer continues. 24 patients have been entered onto study and 17 of 23 patients who have completed therapy have achieved a complete remission. The projected median survival is greater than 2 years and 13 of 23 patients are alive and free of cancer progression with a median follow up of 18 months. One patient has been hospitalized for combined modality pneumonitis.</p> <p>A phase I trial using dihydrolenerone, an agent identified as being active against human lung cancer by the human tumor colony forming assay (HTCFA) has been initiated. Twenty-nine patients have been studied at 6 dosage levels. The principle side effects have been somnolence and hypotension in all patients. Four patients have had to stop therapy because of somnolence and none because of hypotension. Preliminary pharmacokinetic determinations show peak absorption at 3 hours and that level vary less than 50% over the 12 hours dosing interval. There have been no objective responses to date.</p> <p>From this studies we conclude that the HTCFA has identified a compound with novel side effects and the maximum tolerated dose has been reached at 50 mg per square meter.</p>																		



## PROJECT DESCRIPTION

## In Vitro Drug Testing for Limited SCLC and Phase I Drug Development

Professional Staff:

PI:	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
Others:	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	John D. Minna, MD	Chief	NCI-NMOB

Collaborating Branches:

Eli Glatstein, MD, Richard Deming, MD, Radiation Oncology Branch; John Strong, PhD, Robert Parker, PhD, Biological Chemistry Branch and Hoo Geung Chun, MD, Investigational Drug Branch

Objectives:

1. Determine the frequency with which adequate tumor tissue can safely be obtained and drug sensitivity data determined.
2. Determine the response rate, toxicity, and survival of limited stage small cell lung cancer patients treated with VP/PLAT, simultaneous twice a day chest radiotherapy, and chemotherapy based on in vitro drug testing or a standard regimen (VAC).
3. Determine changes in drug sensitivity when tumor is obtained pre and post-treatment from the same patient.
4. Determine the side effects and maximum tolerated dose of dihydrolenerperone.
5. Determine the pharmacokinetics of orally administered dihydrolenerperone.
6. Determine the activity of dihydrolenerperone within the confines of a Phase I trial.
7. Determine the correlation between the in vitro determined activity of dihydrolenerperone and the patient response.

Methods:

1. Small cell lung cancer patients undergo staging
2. Limited stage patients undergo surgical biopsy of tumor tissue
3. Induction with 12 weeks of VP-16/Plat with concomitant 150 RAD twice a day radiotherapy to 4500 RAD over 19 days.
4. Patients with in vitro drug sensitivity data receive an additional 12 weeks of the in vitro best regimen, patients with no in vitro data receive 12 weeks of a standard vincristine, doxorubicin, and cyclophosphamide regimen.

5. Patients are followed for survival and toxicity.
6. Small cell lung cancer patients failing conventional combination chemotherapy and non-small cell lung cancer patients for whom no curative therapies are available are identified for Phase I drug trial.
7. Patients are treated orally twice a day for 28 days with dihydrolenperone and observed for toxicity.
8. Patients with tumor tissue available have in vitro testing with DHLP

#### Major Findings:

#### The Limited Stage Small Cell Lung Cancer Trial Administering VP 16 and Cisplatin Plus B.I.D. Chest Radiotherapy has a Decreased Rate of Pulmonary Toxicity and the Preliminary Survival Data shows a Prolognation of Median Survival

Between 7/86 and 6/89, 24 untreated patients (pts) with LTD stage SCLC were entered onto a combined modality study. 18 were male, 6 female, 3 were PS 0, 19 PS 1, and 2 PS 2. The median age was 57 (range 34-71). Pts were treated with VP 80 mg/m<sup>2</sup> d1,2,3,27,28,29, PLAT 80 mg/m<sup>2</sup> d1,27 with concurrent chest RT 150 cGy bid Mon-Fri d5-24. Patients then received 2 additional cycles of VP/PLAT followed by 4 cycles of either CAV or individualized chemotherapy based on in-vitro drug sensitivity testing. 23 pts have completed therapy and are evaluable for response. 17 had a CR and 6 a PR, overall 100%. Ten of the patients who responded have progressed. 3 progressed in the CNS, 5 outside the chest, and 2 within the RT portal. 13 patients are alive and with no evidence of cancer. The median follow-up is now 18 mo (range 4-32). The actuarial survival is 90% at 1 year and 65% at 2 years. There have been no treatment related deaths. This regimen is well tolerated, associated with minimal toxicity, and the pt survival data are encouraging.

#### Phase I Trial of Dihydrolenpoerone, A Novel Compound Active Against Lung Cancer Identified by the Human Tumor Colony Forming Assay

The phase I and pharmacokinetic study of dihydrolenperone was approved to enter patients in January of 1986. Twenty-nine lung cancer patients have been entered to date and have completed 27 courses of dihydrolenperone. The initial dose was 10mg/m<sup>2</sup> orally twice a day for 28 days. The dosage has been escalated to 20mg/m<sup>2</sup>, 30mg/m<sup>2</sup>, 40mg/m<sup>2</sup>, 50mg/m<sup>2</sup>, 60mg/m<sup>2</sup> b.i.d. and has recently dropped back to 50mg/m<sup>2</sup> because of excessive toxicity. The prominent side effects have been somnolence and hypotension in all patients. The hypotension observed in the first two patients treated with 20mg/m<sup>2</sup> was 70/50 when the patients were standing. This prompted us to alter the loading schedule with dihydrolenperone so the dosage was increased 10mg/m<sup>2</sup> twice daily until the target dosage is reached. The hypotension with this schedule has been more tolerable. Somnolence has been noticeable in all patients with more pronounced somnolence in patients taking narcotics. Four patients discontinued the drug because of somnolence. One patient who was taking methadone for pain caused by tumor involving the liver

discontinued taking dihydrolenperone because of somnolence causing him to sleep more than half the day. Another patient discontinued the DHLP and her symptoms resolved. One other additional patient developed rather severe depression while taking the drug with a flat affect and inability to initiate activities. This developed over the Christmas and New Years holidays. He finished the course of therapy and the depression continued after he stopped the drug. He is currently on Elavil therapy.

Two additional patients did not complete their prescribed courses of dihydrolenperone. One patient developed nausea and vomiting and his drug was stopped. He died within 3 weeks and his autopsy showed tumor constricting his small intestine leading to an intestinal obstruction. The other patient died suddenly two days after his hospital discharge after starting dihydrolenperone. Autopsy showed a massive pulmonary embolus as a cause of death. There has been no observable hematologic, pulmonary, renal, or hepatic toxicity at the dosage levels studied. The pharmacokinetic studies by John Strong of the Biologic Chemistry Branch using HPLC have demonstrated that peak absorption approximately 3 hours after the oral dosage. In addition the twice a day schedule gives drug levels fluctuating less than 50% during the day. The determination of a half life awaits additional studies at higher dosage levels where the drug can be measured more easily. Twenty two different patients have completed at least one course of therapy and are available for analysis of response to therapy. Ten patients had no response to therapy and 12 developed progressive disease. We plan to continue to administer DHLP to an additional two patients and complete the phase 1 trial.

#### Publications:

1. Johnson BE, Steinberg SM, Phelps R, Edison M, Veach SR, Ihde DC. The increasing proportion of women entered into small cell lung cancer clinical trials and its potential effect on outcome. *Am J Med* 1988;85:194-196.
2. Johnson BE, Glatstein E, Ihde DC. The National Cancer Institute experience with early and late effects of combined modality therapy in small cell lung cancer. *Antibiot Chemother* 1988;41:210-212.
3. Ihde DC, Seifter EJ, Johnson BE, Glatstein E. Review of concurrent chemotherapy/radiotherapy combinations in small cell lung cancer. *Antibiot Chemother* 1988;41:92-95.
4. Gazdar AF, Russell EK, Oie HK, Linnoila RI, Steinberg SM, Ghosh BC, Cotelingam JD, Johnson BE, Minna JD, Ihde DC. A prospective clinical trial of individualized chemotherapy and prediction of chemotherapeutic response based on in vitro drug sensitivity testing in extensive stage small cell lung cancer. *Annals Int Med*, submitted.
5. Johnson BE, Grayson J, Makuch RW, Linnoila I, Anderson M, Cohen MH, Glatstein E, Minna JD, Ihde DC. Ten year survival of patients with small cell lung cancer treated with combination chemotherapy with or without irradiation. *J Clin Oncol*, submitted.

6. Johnson BE, Petronas N, Hayes W, Grayson J, Becker B, Gress D, Rowland J, Anderson A, Edison M, Glatstein E, Ihde DC, Frank J. Follow-up of neurologic, computed cranial tomography, and magnetic resonance imaging abnormalities in 6-13 year survivors of small-cell lung cancer. J Clin Oncol, submitted

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-CM-06597-03 NMOB

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Non Small Cell Lung Cancer Therapy Project

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Other:	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Herbert Oie, PhD	Research Biologist	NCI-NMOB
	Edward Russell	Research Biologist	NCI-NMOB
	Mae Jean Englee	Biology Lab Technician	NCI-NMOB
	Sandra Jensen	Biology Lab Technician	NCI-NMOB
	Henry Stevenson, MD	Senior Investigator	NCI-NMOB

## COOPERATING UNITS (if any)

Anatomic Pathology, NHBETH (J. Cottingham); Pulmonary Medicine, NHBETH (T. Walsh); Thoracic Surgery, NHBETH (J. Nesbitt); Radiation Oncology Branch, Surgery Branch, (R. Deming); Clinical Oncology Program

## LAB/BRANCH

NCI-Navy Medical Oncology Branch

## SECTION

Biotherapy

## INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

## TOTAL MAN-YEARS:

3.0

## PROFESSIONAL:

2.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A primary objective of this Branch is to improve the state-of-the-art in the therapy of lung cancer. In the past, this Branch had focused this effort in the study of small cell lung cancer. With the advances both in the therapy of the small cell patients as well as in the study of small cell lung cancer biology, we decided to generalize the Branch effort to include the systemic evaluation of non-small cell lung cancer. The vehicle for this pilot study of the feasibility and value of using in vitro criteria to select therapy for patients with metastatic non-small cell lung cancer.

## PROJECT DESCRIPTION

## Non Small Cell Lung Cancer Therapy Project

Professional Staff:

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Other:	Ilona Linnoila, MD	Pathologist	NCI-NMOB
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	Henry Stevenson, MD	Senior Investigator	NCI-NMOB

Collaborating Branches:

Anatomic Pathology, NHBETH (J. Cottingham); Pulmonary Medicine, NHBETH (T. Walsh); Thoracic Surgery, NHBETH (E. Woods); Radiation Oncology Branch, Surgery Branch, (J. Grayson); Clinical Oncology Program

Objectives:

1. To improve therapy of non small cell lung cancer by selecting chemotherapy on the basis of in vitro analyses, both of drug sensitivity and neuroendocrine markers. To use that protocol as a vehicle for the in-depth study of small cell lung cancer biology.
2. Pilot study to evaluate if patients treated on the basis of their tumor cells' in vitro response to a panel of chemotherapeutic agents have more effective tumor cyoreduction than conventionally treated control patients or historic controls.
3. To determine if non-small cell lung cancer patients with tumors expressing neuroendocrine features characteristic of small cell lung cancer experience natural history more typical of small cell lung cancer.
4. To evaluate our ability to prospectively establish clinical specimens as long-term cell lines.
  - a. Optimizing our ability to grow specimens, especially in developing serum-free media systems.
  - b. Use the computerized clinical and laboratory data bases to correlate

Methods Employed:

1. Clinical trial
2. In vitro drug sensitivity analysis
3. Immunohistochemistry

## 4. Biochemical Marker analysis

## 5. Cell culture

Major Findings:

Since this study opened in April, 1984, over 100 patients have been accrued to this study. We performed an interim analysis on the study.

As a function of protocol design, all patients had tumor tissue come to the laboratory. In several instances, the tumor tissue non-viable due to immersion in formaldehyde, but excellent cooperation between surgeons and pathologists resulted in a better than 95% yield. In order to obtain tissue from as many patients as possible, we frequently obtain tissue from patients undergoing potential curative thoracic resection. We treat only those patients with metastatic disease that is measurable or can be evaluated. Of the 35 patients who already received chemotherapy on this protocol, tumor tissue arrived in the lab was of sufficient size and condition to do at least limited in vitro drug sensitivity analysis in 29%. Some patients have relapsed and died without any chemotherapy (5 patients) and many more are still followed without any evidence of recurrent disease (39 patients). We have established continuous cell lines on 23% of the patients we have evaluated. We project that the frequency of successfully performing in vitro analysis with our current approach may increase to 40% of the total prospective cases. Further refinements of this approach will be necessary to permit this approach to be more generally applicable and we will outline some of the research directions we are pursuing to accomplish this.

These cell lines are a very useful recourse in conducting further experiments to improve the frequency of successful drug sensitivity analysis. First, the initial cell lines derived in the course of non-small cell protocol are being used in validation of another technique of drug sensitivity analysis, the semi-automatic colorimetric assay. This work will be discussed elsewhere in this document, but there are two areas in which the work with this assay impacts on the non-small cell clinical trial. First, this assay requires significantly less operator time to perform, has a more objective mode of analysis, and ultimately may require a smaller number of tumor cells for analysis. Due to the efficacy of this technique, we might also be able to achieve the goal of testing combinations instead of single agents in vitro. For these reasons, we are motivated to substitute this assay for the dye exclusion assay, after we determine the degree of comparability between the two assays. Second, we have used this assay to examine the growth factor requirement of small cell lung cancer to optimize a serum-free media system for those cells. We are now ready to extend this approach to non-small cell tumors as it is apparent from our low rate of successfully generating tumor cell lines that our current media systems are suboptimal. Both of these adjustments, a more efficient in vitro assay and a more effective media system, have the potential of improving the biggest shortcoming of this approach, this is, increasing the percentage of cases that we can successfully test for drug sensitivity in vitro.

As discussed previously, the number of patients actually receiving the combinations of drugs selected by the assay as being most active (based on single agent

activity) is small (8 patients). This number will increase since we plan to accrue another 50 patients and as more of the patients, who underwent potentially curative thoracic resection, develop recurrent disease. Nevertheless, the results of the in vitro analysis suggested their tumors would be minimally responsive. The eight predicted most active combinations resulted in only a half log of cell kill in vitro.

70% of the single agents tested with these 8 tumors were resistant by our arbitrary scale (resulting in less than 50% tumor cell kill). None of these patients had an objective tumor response, but their median survival was five months. The survival rate was equivalent to the patients who received empiric etoposide/cisplatin. Since we are still dealing with small numbers of patients, we have not evaluated the two groups for the equivalence of prognostic features, so it is too early to conclude anything about the utility of the in vitro drug selection to see if a trend emerges. This study, which is really a pilot effort, will not definitely answer the questions regarding the clinical value of in vitro drug sensitivity analysis, but it will provide a departure point for constructing subsequent clinical trials to further resolve such issues.

One of the most provocative directions explored in this study is the prospective evaluation of the fate of the subset of non-small cell lung cancer without biochemical features of small cell cancer. We have prospectively analyzed cell tissues obtained in this study for the expression of four biochemical features generally felt to be characteristic of small cell. Based on the previous retrospective work in characterizing these biochemical markers, expected this phenomenon would be present in about 15% of clear cut non-small cell lung cancers. Our hypothesis was that the patients with these tumors would respond to their treatments in a fashion similar to small cell lung cancer patients (i.e. a higher response rate). We were able to do at least one biochemical parameter on 71 of 81 adequate tumors (88%). 11% of these specimens had elevated levels of expression of at least one biochemical marker. Seven non-small cell lung cancer parts with neuroendocrine biochemical features were treated with a combination chemotherapy used extensively in the Branch for small cell lung cancer (cytoxan, methotrexate, CCNU, vincristine, adriamycin, procarbazine). The response rate has been 43% for those seven "neuroendocrine" patients versus 11% for the remaining 28 non-small cell lung cancer patients treated to date on this study with corresponding median survival rate of 9 months versus 6 months. Considerable work has been done with the in vitro characterization of these neuroendocrine non-small cell lung cancer cell lines, especially in regard to their in vitro drug sensitivity. This will be discussed elsewhere in this document.

#### Significance to Biomedical Research and the Program of the Institute:

Lung cancer is the leading cause of cancer mortality in our society. Non-small cell lung cancer which comprises 75% of all lung cancer is universally fatal once it has metastasized. Despite intensive clinical research, no major improvement has occurred in the treatment of disseminated non-small cell lung cancer. To address this the NCI-Navy Medical Oncology Branch has attempted to integrate a systemic effort to study the biology of this cancer in conjunction with an attempt to optimize the best available treatment. This entails testing a patient's tumor tissue in the laboratory for its response to standard chemo-



therapy agents. Based on the <sup>in vitro</sup> result, a combination is constructed that represents the most cytotoxic single agents for a particular patient's tumor.

This approach potentially has general merit in attempting to specifically tailor available treatments to the unique biology of a patient's tumor. This approach also insures tumor tissue comes to our laboratory and is potentially available to be established as a continuous cell line. Over 30 cell lines have already been established in the course of this study and these lung cancer lines comprise an excellent model system for a variety of laboratory investigations.

#### Proposed Course:

Further accrual of patients to the ongoing protocol will continue. More experience with the process of in vitro drug sensitivity testing to select patient therapy is required both to further analysis of its further refine the process as well as allow fuller analysis of its benefit.

Independent validation of the enhanced initial responsiveness to chemotherapy of patients whose tumor expresses neuroendocrine differentiation is required to corroborate the preliminary clinical trial outcome. To accomplish this, collaborations have been developed with two cooperative groups to analyze for the expression of neuroendocrine features from tumor specimens obtained from patients already treated with chemotherapy. The goal would be examine if the correlation over neuroendocrine expression with enhanced responsiveness to chemotherapy. Further associated biological studies will also proceed.

#### Publications:

1. Gazdar AF, Tsai C-M, Park J-G, Ihde DC, Mulshine JL, Carmichael J, Mitchell J, Minna JD. In vitro assays for predicting clinical response in human lung cancer. In: Peters L, Chapman D, eds. Prediction of Tumor Treatment Response. New York: Pergamon Press, in press.
2. Linnoila RI, Mulshine JL, Steinberg SM, Funa K, Matthews MJ, Cotelingham. Neuroendocrine differentiation in endocrine and non-endocrine lung carcinomas. *Am J Clin Path* 1988;90:641-652.
3. Gazdar AF, Hellman L, Israel M, Russell E, Linnoila I, Mulshine JL, Schuller H, Park J-G. Expression of neuroendocrine cell markers L-Dopa decarboxylase chromogranin a and dense core granules in human tumors of endocrine and non-endocrine origin. *Cancer Res* 1988;48:4078-4082.
4. Tsai C-M, Gazdar AF, Venzon DJ, Steinberg SM, Dedrick RL, Mulshine JL, Kramer BS. Lack of in vitro synergy between etoposide and cis-diamminedichloroplatinum (II). *Cancer Res* 1989;49:2390-2397.
5. Lai S-L, Goldstein LJ, Gottesman MM, Pastan I, Tsai C-M, Johnson BE, Mulshine JL, Ihde DC, Kayser K, Gazdar AF. MDR1 gene expression in lung cancer tumors and cell lines. *J Natl Cancer Inst*, in press.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 06598-03 NMOB																					
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COOPERATING UNITS (if any) Radiation Oncology Branch, (E. Glatstein); Nuclear Medicine, Clinical (J. Carrasquillo); FCRF (J. Mayo); Southern Research Institute (W.R. Laster); Investigational Drug Branch, CTEP (M. Christian, D. Hoth)																							
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TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.0	OTHER: 1.5																					
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The efforts of this Branch has been central to the recognition of gastrin releasing peptide as an autocrine growth factor for small cell lung cancer. Dr. Cuttitta developed a monoclonal antibody (2A11) to the active portion of that peptide and demonstrated that the immunoglobulin could block the mitogenic effect of GRP in vitro and in vivo. We have recently, in collaboration with Hybritech, Inc. (San Diego, CA), initiated a clinical trial to test whether one can control autocrine mediated malignant proliferation of small cell lung cancer using a monoclonal antibody. Our Branch has a long standing interest in the role of growth factors in cancer, so that information from 2A11 antibody clinical trial could be a foundation from subsequent anti-growth factor trials.</p> <p>A recent publication outlined the diagnostic application of lung associated monoclonal antibodies derived at this Branch for use in the early detection of lung cancer. We have patented the method for this approach with collaboration from John Hopkins and in conjunction with the Lung Cancer Study group will proceed to rapidly follow up on this critical area.</p>																							

## PROJECT DESCRIPTION

## Diagnostic and Therapeutic Clinical Trials with Monoclonal Antibodies - Part I

Professional Staff:

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Frank Cuttitta, PhD	Res Assoc Prof NCI-USUHS	NCI-NMOB
	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
	Barnett Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-NMOB
	Ingalill Avis, RN	Biologist	NCI-NMOB

Collaborating Branches:

Radiation Oncology Branch, (E. Glatstein); Nuclear Medicine, Clinical (J. Carrasquillo); FCRF (J. Mayo); Southern Research Institute (W.R. Laster); Investigational Drug Branch, CTEP (M. Christian, D. Hoth)

Objectives:

1. To study the pharmacokinetics of monoclonal antibody delivery, attempting to maximize delivery of antibody to tumor involve sites.
2. To study methods of radiolabeled monoclonal antibody imaging as a staging tool in evaluating patients with cancer.
3. To determine if monoclonal antibody can be used to block growth factor stimulated tumor proliferation.
4. To study tumor cells to identify other growth factors which are potential targets for immunomolecular regulation.

Methods Employed:

1. Radionuclide Imaging
2. Immunohistochemistry/immunocytochemistry
3. Radioimmunoassay
4. Radioautography

Major Findings:

A. This Branch was involved in an early monoclonal antibody therapy trials in cutaneous T-cell lymphoma to determine if the monoclonal antibody could mediate cytoreduction by enhancing immune clearance of malignant T-cell. This trial failed to demonstrate significant therapeutic benefit, but did provide a vehicle for successful diagnostic imaging studies. The initial diagnostic imaging

studies were developed by Paul Bunn, M.D., and continued by Dr. Mulshine. The intravenous delivery of  $\text{In}^{111}$  labeled T101 has resulted in the highest percent of tumor targeting achieved as of the time of its reporting. This localization efficiency was further therapeutically improved after regional delivery via the lymphatics of subcutaneously delivered  $\text{In}^{111}$  labeled T101. Further studies included a comparison of quality imaging  $\text{In}^{131}$  T101 versus  $^{111}\text{Tl}$  T101. In this study, the  $\text{In}^{111}$  conjugate was clearly superior. This work now proceeds to further analysis of specificity of targeting by using an isotopically matched  $\text{In}^{111}$  T101-control antibody in sequential scanning studies with  $\text{In}^{111}$  T101. Information generated in the course of these studies include enhanced understanding of the pharmacology of antibody targeting, the immunogenicity of administered mouse immunoglobulin, and the efficiency of regional delivery techniques. These studies collectively provide the basis for proceeding with the radiolabeled T101 therapy trial which is discussed separately. Efforts have been productive, both in terms of published manuscripts and in developing useful collaborations with other investigators at the Clinical Center engaged in clinical research with monoclonal antibodies.

B. Small cell lung cancer has been extensively studied both at this Branch and elsewhere as a model of a neuroendocrine tumor. Small cell lung cancer has already been reported to produce over 25 different peptide hormone products. Recently, workers from our lab sequenced the gene for GRP from small cell lung cancer. A family of previously unknown peptides synthesized from open reading frames found on the GRP gene. Hetero anti-sera were developed to the three GRP gene associated peptides (G-Gap peptides). By several assays, immunologic evidence of expression of these three distinct products was documented in both small cell tumors and in fetal tissues. These facts suggest that despite the already known numbers of peptide products of small cell lung cancer, there may be a considerably larger number of small cell tumor products. With the rapid development of many areas of biotechnology, the techniques may now be available to begin systematically evaluating the total peptide synthetic capabilities of small cell.

C. We are interested in elucidating and additional new peptide products of small cell, we propose to focus on those peptide products that possess mitogenic capabilities. To facilitate this, we have invested considerable time in validating a semi-automatic colorimetric assay for evaluation of growth factor effects. The parameters to evaluation for such an application are considerably different than the conditions for the assay as reported by Carmichael and others from our Branch. The advantage of this assay is that it provides the exceedingly efficient assay to monitor for growth stimulatory effects, which will be essential when screening large numbers of purified fractions generated in typical HPLC purification efforts.

D. Using the semi-automatic colorimetric assay, we have already demonstrated the mitogenic effect of insulin-like growth factor-I (IGF-I) on small cell lung cancer cell lines. We have further demonstrated that this effect can be blocked by a monoclonal antibody specific for the anti-IGF-I receptor.

We have studied the biology of IGF-I in small cell and it appears to be an attractive candidate to target for a therapy approach similar to the anti-GRP

monoclonal antibody trial. In thinking about GRP and a candidate for immunotherapy, the limited role this molecule plays in normal adult physiology potentially permits one to completely block this peptide effect without lethal consequences. The situation with IGF-I may not be similar as this molecule plays a more obvious role in normal adult physiology. Although that might not prevent us from exploring the same type of anti-autocrine factor strategy we employed in the anti-GRP trial, it did provoke us to consider approaches.

E. Many investigators have suggested that cancer can be thought of as a re-expression of normal embryonic and fetal developmental processes. An extrapolation is that autocrine type stimulation may be an important developmental mechanism. If so, such autocrine proliferation should be controlled through some signaling mechanism to allow for the uniform development of a fetus. In cancer, autocrine proliferation proceeds unabated either because of the regulatory signal. We tested to see if the stimulation of small cell lung cancer mediated by IGF-I could be inhibited by glucagon, a normal antagonist of IGF-I activity. Of interest, at a concentration of 10 g/ml, glucagon inhibits the growth enhancement of exogenous effect of IGF-I in other cell lines. In addition, we are attempting to define the mechanism mediating the inhibitory effect in the cell lines responsive to glucagon.

#### Significance to Biomedical Research and the Program of the Institute:

These studies have two goals: First to complete the ongoing trial which represent a first effort to establish the clinical utility of monoclonal antibody based imaging and treatment approaches; Second, we have design ongoing in vitro analysis in conjunction with the clinical trials as well as other laboratory investigations to develop second generation biological trials which lend to more effective therapeutic control of malignant proliferation.

#### Proposed Course:

The Phase I component of the anti-GRP trial is ongoing and will be extended to Phase II when appropriate. Clinical trials with the other antibodies will also continue with the goal of moving to radionuclide conjugate therapy using monoclonal antibodies in cutaneous T-cell lymphoma and lung cancer. Further work will continue to develop a feasible approach to block IGF-I stimulation of lung cancer. Dr. Cuttitta is generating antibodies against synthetic peptides from various portions of prepro IGF-I and the IGF-I receptor. Using either an available reagent or Branch derived product, we will do further in vivo work to block IGF-I stimulation. This work may lead us to a clinical trial in a similar fashion to the anti-GRP monoclonal antibody trial. Ultimately, one may choose to block both GRP and IGF-I stimulation. Careful analysis of early growth factor therapy trials may provide insight as to how best to proceed.

#### Publications:

1. Nakanishi Y, Cuttitta F, Kasprzyk PG, Avis I, Steinberg SM, Gazdar AF, Mulshine JL. Growth factor effects on small cell lung cancer cells using a colorimetric assay: Can a transferrin-like factor mediate autocrine growth. *Exper Cell Biol* 1988;56:74-85.

2. Nakanishi Y, Mulshine J, Kasprzyk P, Natale RB, Maneckjee R, Avis I, Treston AM, Gazdar AF, Minna JD, Cuttitta F. Insulin-like growth factor I can mediate autocrine proliferation of human small cell lung cancer cell lines in vitro. *J Clin Invest* 1988;82:354-359.
3. Treston AM, Kasprzyk PG, Covey T, Lee ED, Henion J, Yergery A, Cuttitta F, Mulshine JL. Applications of mass spectrometric to the identification of novel peptide hormones involved with lung cancer biology. In Rosen ST, Mulshine JL, Cuttitta F, Abrams PG, eds. *Biology of Lung Cancer*. New York: Marcel Dekker, 1988;91-110.
4. Kasprzyk PG, Cuttitta F, Treston AM, Avis I, Nakanishi Y, Wong H, Walsh J, Mulshine JL. Consideration of the chemistry of solid-phase matrix interactions leads to improved quantitation of neuropeptides. *Annals NY Acad Sci* 1988;547:41-53.
5. Kasprzyk PG, Cuttitta F, Avis I, Nakanishi Y, Treston A, Wong H, Walsh JH, Mulshine JL. Solid phase peptide quantitation assay using labelled monoclonal antibody and gluteraldehyde fixation. *Anal Biochem* 1988;174:224-234.
6. Tockman MS, Gupta PK, Myers JD, Frost JK, Baylin SB, Gold EB, Mulshine JL. Sensitive and specific monoclonal antibody recognition of human lung cancer antigen on preserved sputum cells: A new approach to early lung cancer detection. *J Clin Oncol* 1988;6:1685-1693.
7. Mulshine JL, Avis I, Treston AM, Mobley C, Kasprzyk PG, Carrasquillo JA, Larson SM, Nakanishi Y, Merchant B, Minna JD, Cuttitta F. Clinical use of a monoclonal antibody to bombesin-like peptide in patients with lung cancer. *Proceedings of the First International Symposium of Bombesin-Like Peptides*. NY Acad Sci 1988;547.
8. Rosen S, Mulshine JL, Cuttitta F, Abrams P, eds. *Lung Cancer Biology*. New York: Marcel Dekker, 1988.
9. Trepel JB, Moyer JD, Cuttitta F, Frucht H, Coy DH, Natale RB, Mulshine JL, Jensen RT, Sausville EA. A novel bombesin receptor antagonist inhibits autocrine signals in a small cell lung carcinomas cell line. *Biochem Biophys Res Comm* 1988;255:403-410.
10. Cuttitta F, Fedorko J, Gu J, Lebacqz-Verheyden AM, Linnoila RI, Battey JF. Gastrin releasing peptide gene associated peptides (GGAPs) are expressed in normal human fetal lung and small cell lung cancer: A novel peptide family found in man. *J Clin Endocrinol Metab* 1988;67:576-582.
11. Treston AM, Mulshine JL. Beyond transcriptional events. *Nature* 1989;337:406.
12. Reeve JR, Cuttitta F, Vigna SR, Heubner V, Lee TD, Shively JE, Ho F-J, Fedorko J, Minna JD, Walsh JH. Multiple gastrin-releasing peptide gene-associated peptides are produced by a human small cell lung cancer line. *J Biol Chem* 1989;264:1928-1932.

13. Mulshine JL, Natale RB, Avis I, Treston AM, Kasprzyk PG, Nakanishi Y, Sausville EA, Trepel JB, Cuttitta F. Autocrine growth factor and lung cancer. In Hansen H, ed. Basic and Clinical Concepts of Lung Cancer. Boston: Kluwer Academic Publishing. 1989;107-122.
14. Mulshine J, Avis I, Treston AM, Kasprzyk PG, Nakanishi Y, Mobley C, Carrasquillo JA, Larson SM, Merchant B, Cuttitta F. In vivo diagnosis and therapy of human tumors with monoclonal antibodies selection of antibodies and preliminary clinical studies in small cell carcinoma of the lung. Nucl Med Biol 1989;16:159-162.
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16. Nakanishi Y, Cuttitta F, Kasprzyk PG, Treston AM, Avis I, Minna JD, Kleinman HK, Mulshine JL. The effects of growth factors on the in vitro growth of small cell lung cancer as determined in a colorimetric assay. In: Rosen S, Mulshine JL, Cuttitta F, Abrams PG, eds. Tumor Biology. New York: Marcel Dekker, in press.
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<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 06599-03 NMOB
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Supportive Care Project		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
<div style="display: flex; justify-content: space-between;"> <div>             PI: Barnett S. Kramer, MD              Others: Michael Bolger, MD           </div> <div>             Assoc Prof Med NCI-USUHS              Lt Cdr, MD, USN           </div> <div>             NCI-NMOB              NCI-NMOB           </div> </div>		
COOPERATING UNITS (if any) Division of Infectious Diseases, National Naval Medical Center, Bethesda (Walter Carney, Kenneth Wagner, Matthew Pollack); Pediatric Oncology Branch, DCT, NCI (Philip Pizzo)		
LAB/BRANCH NCI-Navy Medical Oncology Branch		
SECTION Clinical Investigations		
INSTITUTE AND LOCATION NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 3.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)		
<p>The primary goal of this group is to investigate the prophylaxis and management of infectious complications of cancer patients undergoing chemotherapy. A recently completed study was one of prophylaxis of infection in neutropenic patients with passive immunization. It was a randomized study of intravenous gammaglobulin (IVIG) versus placebo. Final analysis in 75 patients randomized at the NCI and Naval Hospital, Bethesda, showed no differences between IVIG and placebo in incidence of fever, documented infection, or duration of fever. The subsequent project was a randomized study of monotherapy (ceftazidime) versus ceftazidime plus vancomycin for the initial empiric therapy of febrile cancer patients with very low white blood counts. A total of 129 episodes were analyzed between the two collaborating centers (NCI-NMOB and University of Florida; 36 episodes at NCI-NMOB). The study has been completed. Final analysis showed that single agent ceftazidime was equivalent to initial ceftazidime plus vancomycin in survival and ultimate response, but saved about \$250 in medication costs per course of therapy. Results were presented at the International Congress of Antimicrobial Agents and Chemotherapy (October, 1988).</p>		



PROJECT DESCRIPTION  
Supportive Care Project

Professional Staff:

PI: Barnett S. Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-NMOB
Others: Michael Bolger, MD	Lt Cdr, MD, USN	NCI-NMOB

Collaborating Branches:

Division of Infectious Diseases, National Naval Medical Center, Bethesda (Walter Carney, Kenneth Wagner, Matthew Pollack); Pediatric Oncology Branch, COP, DCT (Philip Pizzo)

Objectives:

1. IVIG Study (Completed since last annual report)
  - a. To examine in a randomized double blinded study whether passive immunization with intravenous gamma globulin (IVIG) is effective as prophylaxis against infection in neutropenic cancer patients.
  - b. To determine the pharmacokinetics of IVIG and correlate with efficacy of IVIG.
  - c. To monitor toxicity of IVIG.

2. Ceftazidime Study

To establish criteria for the addition of vancomycin to initial empiric monotherapy with ceftazidime in the empiric treatment of febrile neutropenic cancer patients.

Methods Employed:

Both of the above studies are prospective and randomized. The IVIG study was double-blinded with a placebo arm using albumin.

Assays for blood immunoglobulin levels are being performed.

Major Findings:

The IVIG Study is completed. An analysis of 75 episodes showed no differences in outcome (incidence of fever, incidence of infection, duration of fever) between IVIG and placebo. Analysis of the recently completed ceftazidime + vancomycin study showed that outcome was equivalent in both treatment arms, but cost was lower in the ceftazidime arm.

Significance to Biomedical Research and the Program of the Institute:

Infection is a major cause of death in cancer patients and is a very common reason for hospitalization. The best therapeutic regimen for febrile neutropenic patients has not yet been established. We were looking for an effective non-toxic regimen.

Proposed Course:

We are presently negotiating with a manufacturer of GM-CSF (granulocyte-monocyte colony stimulating factor) to do a randomized study in cancer patients who become febrile and neutropenic. This study would be performed in collaboration with the Pediatric Oncology Branch of the National Cancer Institute (Drs. Philip Pizzo and Marc Rubin).

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 07250-03 NMOB																		
PERIOD COVERED October 1, 1988 to September 30, 1989																				
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders ) New Drug Discovery Project																				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Barnett S. Kramer, MD</td> <td style="width: 33%;">Assoc Prof Med NCI-USUHS</td> <td style="width: 33%;">NCI-NMOB</td> </tr> <tr> <td>Others: Adi Gazdar, MD</td> <td>Senior Investigator</td> <td>NCI-NMOB</td> </tr> <tr> <td>Bruce Johnson, MD</td> <td>Investigator</td> <td>NCI-NMOB</td> </tr> <tr> <td>Daniel Ihde, MD</td> <td>Prof Med NCI-USUHS</td> <td>NCI-NMOB</td> </tr> <tr> <td>James Mulshine, MD</td> <td>Senior Investigator</td> <td>NCI-NMOB</td> </tr> <tr> <td>Mary Pat Dearing, MD</td> <td>Medical Staff Fellow</td> <td>NCI-NMOB/NCI-IDB</td> </tr> </table>			PI: Barnett S. Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-NMOB	Others: Adi Gazdar, MD	Senior Investigator	NCI-NMOB	Bruce Johnson, MD	Investigator	NCI-NMOB	Daniel Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB	James Mulshine, MD	Senior Investigator	NCI-NMOB	Mary Pat Dearing, MD	Medical Staff Fellow	NCI-NMOB/NCI-IDB
PI: Barnett S. Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-NMOB																		
Others: Adi Gazdar, MD	Senior Investigator	NCI-NMOB																		
Bruce Johnson, MD	Investigator	NCI-NMOB																		
Daniel Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB																		
James Mulshine, MD	Senior Investigator	NCI-NMOB																		
Mary Pat Dearing, MD	Medical Staff Fellow	NCI-NMOB/NCI-IDB																		
COOPERATING UNITS (if any) Radiation Oncology Branch (E. Glatstein); Nuclear Medicine, Clinical Center (J. Carrasquillo); Frederick Cancer Research Program (J. Mayo); Southern Research Institute (W.R. Laster); Investigational Drug Branch, CTEP (M. Christian)																				
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SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)  <p>The primary goal of this group is to identify new agents of potential clinical use in treating solid tumors. A major effort over the past year has been the use of an in vitro assay which may be helpful as a preclinical screening model for antitumor agents. The model has been used to predict the clinical activity of 7 chemotherapeutic agents against 11 human colorectal carcinoma cell lines which have been developed in this branch. Using the model, we have shown that leucovorin enhances the in vitro cytotoxicity of the fluoropyridines versus our panel of colorectal cell lines. A study was also performed to detect possible synergy between etoposide and cisplatin in a panel of 8 human bronchogenic carcinoma cell lines. Extensive analysis revealed no in vitro synergy, a finding at variance with standard feeling.</p> <p>At present, we are involved in several trials of new experimental therapeutic agents: Dihyrolenperone in lung cancer; a radiolabeled monoclonal antibody (<sup>90</sup>yttrium-Tl01) in mycosis fungoides and chronic lymphocytic leukemia; 4-ipomeanol in lung cancer and a Phase I trial of hepsulfam.</p>																				

## PROJECT DESCRIPTION

## New Drug Discovery Project

Professional Staff:

PI: Barnett S. Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-NMOB
Others: Adi Gazdar, MD	Senior Investigator	NCI-NMOB
Bruce Johnson, MD	Investigator	NCI-NMOB
Daniel Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
James Mulshine, MD	Senior Investigator	NCI-NMOB
Mary Pat Dearing, MD	Medical Staff Fellow	NCI-NMOB/NCI-IDB

Collaborating Branches:

Radiation Oncology Branch (E. Glatstein); Nuclear Medicine, Clinical Center (J. Carrasquillo); Frederick Cancer Research Program (J. Mayo); Southern Research Institute (W.R. Laster); Investigational Drug Branch, CTEP (M. Christian)

Objectives:

1. Identification of new compounds for the treatment of solid tumors, especially colorectal carcinoma.
2. Preclinical testing of combinations of drugs to detect synergy.
3. Validation of in vitro chemosensitivity test.
4. Testing new compounds in the clinic for lung and colon cancers.

Methods Employed:

1. In vitro chemosensitivity: MTT assay (a tetrazolium-based colorimetric test for cell viability).
2. Phase I trials of new drugs in cancer (for example, dihydrolenperone and ipomeanol in lung cancer; sulfamic acid).

Major Findings:

1. 5-FU was the only one of 7 drugs tested which we predict would be effective in some of our colorectal cell lines.
2. Leucovorin enhanced the cytotoxicity of 5-FU and of FUDR in 10 of 11 colorectal cell lines tested.
3. The dihydrolenperone study is proceeding (see Dr. Bruce Johnson's Annual Report).
4. The ipomeanol study has opened (2 patients treated to date); the <sup>90</sup>yttrium-Tl01 study has also opened (3 patients treated to date).

Significance to Biomedical Research and the Program of the Institute:

New drug development is a major charge of the National Cancer Institute. The preclinical screening program of the NCI is based upon the MTT assay. It is important to pursue innovative therapies, such as treatment with radiolabeled monoclonal antibodies directed against malignant cells (e.g. <sup>90</sup>yttrium-T101).

Publications:

1. Tsai C-M, Gazdar AF, Venzon DJ, Steinberg SM, Dedrick RL, Mulshine JL, Kramer BS. Lack of in vitro synergy between etoposide and cis-diammine-dichloroplatinum(II). Cancer Res 1989;49:2390-2397.
2. Park J-G, Collins JM, Gazdar AF, Allegra CJ, Steinberg SM, Greene RF, Kramer BS: Enhancement of fluorinated pyrimidine-induced cytotoxicity by leucovorin in human colorectal carcinoma cell lines. J Natl Cancer Inst 1988;80:1560-1564.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 07255-01 NMOB
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biologic Properties of Nuclear Oncogenes and Attempts to Block Their Effects		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI: Michael J. Birrer, MD, PhD Others: Rhoda Alani Richard Rosenberg, MD Hirotooshi Dosaka, MD, PhD	Asst Prof Med NCI-USUHS Howard Hughes Med Scholar Instructor Med NCI-USUHS Visiting Fellow	NCI-NMOB NCI-NMOB NCI-NMOB NCI-NMOB
COOPERATING UNITS (if any) University of California San Diego (Dr. Michael Karin)		
LAB/BRANCH NCI-Navy Medical Oncology Branch		
SECTION Genetics, Molecular Biology and Immunology		
INSTITUTE AND LOCATION NCI, COP, DCT, Naval Hospital, Bethesda, MD 20814		
TOTAL MAN-YEARS: 3	PROFESSIONAL: 3	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Recent developments in molecular biology has led to the identification of specific genetic lesions resulting in either activation or inactivation of key target genes in various tumor systems. These genes, called oncogenes, are involved in various aspects in the regulation of cell growth. It is now critical to understand the precise mechanism by which these genes function so molecular agents ultimately can be derived to alter or repress their effects.</p> <p>We have chosen to explore the biologic and biochemical functions of 2 dominant (L-myc and c-jun) and 1 recessive oncogene (p53). Transcriptional and translational products of L-myc have been characterized and are now being correlated with biologic functions. Ultimately, truncated fragments of this gene will be tested for potential transformation suppression function.</p> <p>Likewise, we have recently described the transforming function of c-jun in mammalian cells and are now mapping this function by deletion mutation. Correlation of this function with other known activities of c-jun, such as transactivation will be done.</p> <p>Finally, recent data suggests that deletion or mutation of the p53 gene is important in the development of lung tumors. In an attempt to address the role of this gene in this tumor system and to explore the possible therapeutic application of gene therapy for this disease, we are attempting to reintroduce a normal p53 gene into lung cancer tumor cells.</p>		

## PROJECT DESCRIPTION AND RESULTS

## Biologic Properties of Nuclear Oncogenes and Attempts to Block Their Effects

PI:	Michael J. Birrer, MD, PhD	Asst Prof Med NCI-USUHS	NCI-NMOB
Others:	Rhoda Alani	Howard Hughes Med Scholar	NCI-NMOB
	Richard Rosenberg, MD	Instructor Med NCI-USUHS	NCI-NMOB
	Hirotoshi Dosaka, MD, PhD	Visiting Fellow	NCI-NMOB

Project #1 The L-myc Proteins and Their Biologic Activities:

The protein products of the L-myc gene have been further characterized. The larger molecular weight species possibly arise from alternative translational initiation sites (including a non-AUG initiation site) and post-translational phosphorylation. These various proteins have been shown to cotransform rat embryo cells with an activated ras gene and are presently being examined for differences in their biologic activities. (Dosaka and Birrer in collaboration with Minna)

To explore and expand the biologic functions of L-myc and compare it to c-myc, the gene was transfected into murine erythroleukemia cells (MEL). When constitutively expressed, L-myc was able to substitute for c-myc in blocking the differentiation of these cells. This suggests that the critical region of myc involved in MEL differentiation are there which are conserved between L-myc and c-myc. (Birrer and Segal)

Project #2 The Transforming Activity of the c-jun Proto-oncogene:

The transforming activity for the c-jun proto-oncogene was established by demonstrating the cotransforming activity of this gene in combination with an activated ras gene in rat embryo cells. Further, it was shown that c-jun can transform an immortalized rat fibroblast cell line Rat-1a as a single gene. This demonstrates that no mutational activating event is required for c-jun to transform mammalian cells. (Birrer, in collaboration with Schutte and Minna)

To elucidate the mechanism of transforming activity of c-jun we have undertaken a mutation/deletion study of the gene. Presently, the transforming activity of the gene maps to two highly conserved regions in the gene, one of which contains the DNA binding domain. Preliminary experiments map these transforming domains to those required for transactivation. Further, in isolating these mutants, some non-transforming ones were found to inhibit the transforming activity of the full length gene, hence displaying a "dominant-negative" phenotype. We are presently characterizing these for their biologic and biochemical properties. (Alani, Rosenberg, Dosaka and Birrer)

Project #3 Gene Therapy in Lung Cancer Cells:

Experiments over the last several years has elucidated several key genetic events in the genesis of lung cancer. In a direct attempt to address the

importance of these genetic lesions and the potential therapeutic advantage of manipulation of these lesions, we have undertaken to design molecular agents with growth regulating effects and deliver them to lung cancer cells in vitro. The mode of delivery includes traditional transfection techniques and newly designed retroviruses. The potential therapeutic agents include truncated forms of the myc family of proto-oncogenes, deletion mutants of c-jun all of which in other systems appear to have dominant-negative phenotypes and most recently the nuclear proto-oncogene p53. (Rosenberg, Alani, Dosaka and Birrer)

p53 has been reported to be frequently deleted or mutated in colon. We have recently screened a large number of lung cancer cell lines and tumors for p53 expression and found that the majority have a mutated or deleted p53 gene suggesting it plays an important role in the development of a lung neoplasm. To further explore the role of p53 in lung cancer we have inserted the normal p53 gene into an inducible eukaryotic expression vector and transfected it into several lung cancer cell lines include one which is homozygously deleted for the gene. Preliminary analysis suggests that the gene has a potent growth suppression effect on lung cancer cell lines. (Rosenberg, Takahashi, Birrer in collaboration with Minna)

#### Publications:

1. Birrer MJ, Young RC. Differential diagnosis of jaundice in lymphoma patients. *Semin Liver Dis* 1987;7:269-277.
2. Birrer MJ, Minna JD. Molecular genetics of lung cancer. *Semin Oncol* 1988;15:226-235.
3. Birrer MJ, Raveh L, Dosaka H, Segal S. A transfected L-myc gene can substitute for c-myc in blocking murine erythroleukemia differentiation. *Mol Cell Biol* 1989;9:2734-2737.
4. Schutte J, Minna JD, Birrer MJ. Deregulated expression of human c-jun transforms primary rat embryo cells in cooperation with an activated c-Ha-ras gene and transforms Rat-1a cells as a single gene. *Proc Natl Acad Sci USA* 1989;86:2257-2261.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 07256-01 NMOB
PERIOD COVERED October, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanisms of Oncogene Action in Tumorigenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Frederic J. Kaye, MD Others: Sarah Barksdale Ray Buchmann J. William Harbour Jean Gerster Robert Kratzke, MD	Asst Prof Med-NCI/USUHS Howard Hughes Med Scholar SRTP Howard Hughes Med Scholar Research Assoc NCI-USUHS Medical Staff Fellow	NCI-NMOB NCI-NMOB NCI-NMOB NCI-NMOB NCI-NMOB NCI-NMOB
COOPERATING UNITS (if any) Whitehead Institute, Boston, MA (J. Horowitz, R. Weinberg); Mass. General Hospital, Boston, MA (S. Friend)		
LAB/BRANCH NCI-Navy Medical Oncology Branch		
SECTION Genetics, Molecular Biology, and Immunology		
INSTITUTE AND LOCATION NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814		
TOTAL MAN-YEARS: 5	PROFESSIONAL: 4	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>We have undertaken a study to identify the critical genetic events in the pathogenesis of lung cancer. We have focused our efforts on two model systems. The first project studies the mechanism and implication of inactivation of the retinoblastoma (Rb) gene in lung cancer. Our recent findings have demonstrated that essentially all small cell lung cancer tumors have abnormalities targeted to this gene. This dramatic finding is being pursued with an effort toward reverting tumorigenicity by reintroduction of the wild-type Rb gene. These experiments may ultimately prove to be of great importance in the diagnosis, treatment and prevention of this common adult tumor.</p> <p>The second project involves the study of myc gene activation in lung cancer. Myc gene activation has been associated with a variety of human cancers although the nature of this association has remained unclear. We have undertaken a study of the regulation of the L-myc gene to identify the factors that contribute to its deregulation which is observed in over 50% of SCLC tumors.</p>		

## PROJECT DESCRIPTION

## Mechanisms of Oncogene Action in Tumorigenesis

Project #1 Role of the Retinoblastoma Gene in the Pathogenesis of Human Cancer

PI:	Frederic J. Kaye, MD	Asst Prof Med NCI-USUHS	NCI-NMOB
Others:	J. William Harbour	Howard Hughes Med Scholar	NCI-NMOB
	Jean Gerster	Research Assoc NCI-USUHS	NCI-NMOB
	Robert Kratzke, MD	Medical Staff Fellow	NCI-NMOB

We have demonstrated acquired structural mutations within the retinoblastoma gene (Rb) in 20% of small cell lung cancer (SCLC) and pulmonary carcinoid tumors and absent mRNA expression in 60% of SCLC specimens. The frequency of these abnormalities is analogous to that previously reported for retinoblastoma and its associated mesenchymal tumors and these findings in SCLC have now been independently confirmed by two other groups in the field.

We now wish to address two critical questions:

- A. Can we revert tumorigenicity in SCLC by reintroducing the Rb gene and can we use this information to implement preventive or therapeutic strategies?
- B. What is the role of the Rb gene in normal cellular physiology and how does its inactivation result in tumorigenesis?

Question A: Transfection of the Rb gene into SCLC cell lines.

In collaboration with S. Friend (Boston, MA), we obtained a clone for the full-length open reading frame of the Rb gene. We have constructed expression vectors and have begun transfection experiments into SCLC lines. Stable transformants will be analyzed for production of the exogenous Rb construct and tested for loss of tumorigenicity.

Question B: Identification and characterization of mutant Rb proteins in SCLC.

Although 40% of SCLC tumors express a normal sized mRNA, we have now shown that the majority of these transcripts are defective and result in absent or mutant Rb protein.

Therefore, in excess of 90% of SCLC tumors studied to date have evidence for Rb inactivation. In collaboration with J. Horowitz and R. Weinberg (Boston, MA), we have identified several cell lines with mutant proteins and have characterized the molecular defects that generated these mutants. In particular, the phosphorylation state of the Rb protein is presumed to play an important role in modulating the effect of this product on cellular proliferation. We have identified one cell line with an inability to phosphorylate its Rb protein. Our lab is currently involved in an effort to identify the subtle mutation which generated this defect in order to define this phosphorylation domain.

In addition to the above, we have investigated other related tumor types for evidence of inactivation of the Rb gene, and found that certain small cell cancers of extrapulmonary origin (such as primary small cell of the prostate or brain) have structural mutations within this recessive oncogene. We have also undertaken a comprehensive study of non-SCLC tumors for evidence of subtle abnormalities in this gene. (In collaboration with J. Minna, S. Segal, A. Gazdar, Y. Kim)

#### Publications:

1. Harbour J, Lai S, Whang-Peng J, Gazdar A, Minna JD, Kaye F. Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. Science 1988;241:353-357.
2. Harbour J, Johnson B, Gazdar A, Minna JD, Kaye F. Inactivation of the retinoblastoma gene in small cell tumors of extrapulmonary origin. Proc Natl Acad Sci USA, submitted.
3. Horowitz J, Park S, Kaye F, Minna JD, Weinberg R. Frequent loss and mutation of the retinoblastoma protein in retinoblastoma, small cell lung carcinoma, and bladder carcinoma cells. In preparation.
4. Kaye F, Barksdale S, Harbour JW, Minna JD. Oncogenes in lung cancer. In preparation.

#### Project #2 Mechanisms of L-myc Activation in Lung Cancer

PI:	Frederic J. Kaye, MD	Asst Prof Med-NCI/USUHS	NCI-NMOB
Others:	S. Barksdale	Howard Hughes Med Scholar	NCI-NMOB
	R. Buchmann	STRP	NCI-NMOB
	R. Kratzke, MD	Medical Staff Fellow	NCI-NMOB

Our goals are to address the following questions:

- A) How is the L-myc oncogene regulated?
- B) What are the mechanisms of myc deregulation in lung cancer?

The L-myc gene is frequently deregulated, with or without gene amplification, in small cell lung cancer (SCLC). In order to understand the mechanism of myc activation in these tumors, we have undertaken a study of the transcriptional regulation of the L-myc gene in SCLC. Using reporter gene (CAT) assays, exonuclease mapping, and mobility shift assays, we have now defined the L-myc promoter and have identified at least 8 specific DNA: nuclear protein interactions within the upstream sequence and exon 1. Two of these interactions represent binding of Spl, a common transcriptional activating protein. The remaining DNA: protein interactions appear to be novel binding sites. We are currently correlating the functional activity of these transacting factors.

We are especially interested in DNA-binding proteins within exon 1 since we observed promoter activity in this exon and also because this region is presumed to contain the signals for the "block to transcription elongation" mechanism. This "transcription block" is reported to be the principle mechanism of myc gene down-regulation during the differentiation process. Consequently, we have identified three specific DNA: protein binding sites within exon 1. One of these transacting factors binds to an 8-bp sequence that is repeated in several human genes (including promoter elements of histone genes, insulin receptor genes, CSF-1 gene and metallothionein genes) and viral genes of the Herpes family (HSV I and II, CMV, and EBV). We believe this represents a binding site for a new family of transcription factor. We have mutated this site and are studying its functional activity. We also plan to purify these nuclear proteins that bind to c-myc exon 1 using affinity chromatography techniques.

#### Publications:

1. Kaye F, Battey J, Nau M, Brooks B, Seifter E, DeGreve J, Birrer M, Sausville E, Minna J. Structure and expression of the L-myc gene reveal a complex pattern of alternative mRNA processing. *Mol Cell Biol* 1988;8:186-195.
2. Barksdale S, Buchmann R, Kaye F. An 8-bp sequence in L-myc exon 1 is identical to a regulatory element of the histone H4B gene. *Science*, submitted.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 CM 07257-01 NMOB
<b>PERIOD COVERED</b> October 1, 1988 to September 30, 1989		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)</b> Molecular Biology of Erythroleukemia and F9 Teratocarcinoma Cell Differentiation		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</b>		
<b>PI:</b> Shoshana Segal, PhD	Assoc Prof Med NCI-USUHS	NCI-NMOB
<b>Others:</b> Matia Bar-Ner, PhD Lora Messing	Fogarty Visiting Fellow Research Assoc NCI-USUHS	NCI-NMOB NCI-NMOB
<b>COOPERATING UNITS (if any)</b> Hematology, Oncology Section University of Pennsylvania		
<b>LAB/BRANCH</b> NCI-Navy Medical Oncology Branch		
<b>SECTION</b> Molecular Biology of Differentiation		
<b>INSTITUTE AND LOCATION</b> NCI, DCT, COP, Naval Hospital Bethesda MD, 20814		
<b>TOTAL MAN-YEARS</b> 3.0	<b>PROFESSIONAL:</b> 3.0	<b>OTHER:</b> 0
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)</b> <p>Cellular differentiation is a complex process for which the molecular mechanisms are poorly understood. How changes in growth potential are related to expression of the differentiated phenotype is at present unknown. We have focused our attention on questions such as the role of oncogenes in the differentiation process of murine erythroleukemia (MEL) and F9 teratocarcinoma cell lines. We were able to demonstrate that in both cell lines, high levels of expression of a transfected c-myc gene blocks HMBA, DMSO or Retinoic Acid (RA) induced differentiation.</p> <p>Based on these findings and the published reports on the homology between C, N, and L-myc protooncogenes, we investigated the ability of the related L-myc gene to substitute for c-myc in blocking MEL differentiation. Our results clearly indicated that constitutive high levels of transfected L-myc mRNA block inducer mediated differentiation of MEL cells. Similar results were obtained recently in our laboratory with N-myc transfectants. These studies strongly suggest that down regulation of c-myc expression in these cell lines is a necessary event for terminal differentiation. We are using a large number of deletion and insertion mutants of the c-myc gene for mapping the region(s) responsible for its apparent critical role in MEL and F9 teratocarcinoma cell differentiation. In addition, we have constructed a subtractive cDNA library from induced and uninduced MEL cells for the purpose of identifying and cloning genes involved in hematopoietic terminal differentiation.</p>		

## PROJECT DESCRIPTION

## Molecular Biology of Erythroleukemia and F9 Teratocarcinoma Cell Differentiation

PI: Shoshana Segal, PhD	Assoc Prof Med NCI-USUHS	NCI-NMOB
Others: Matia Bar-Ner, PhD	Fogarty Visiting Fellow	NCI-NMOB
Lora Messing	Research Assoc NCI-USUHS	NCI-NMOB

Collaborating Branches:

Hematology, Oncology Section University of Pennsylvania

Objectives:

1. To study the role of C, N, and L-myc protooncogenes in growth and differentiation of MEL and F9 teratocarcinoma cells.
  2. To identify and map regions on the c-myc gene essential for differentiation.
  3. To study mechanisms and genes involved in hematopoietic and F9 teratocarcinoma cell differentiation.
- A. Members of the Myc Family Block Chemically Induced Differentiation of MEL and F9 Teratocarcinoma Cells.

MEL and F9 teratocarcinoma cells express high levels of the c-myc protooncogene, however shortly after the addition of inducer (HMBA, DMSO, RA) a sharp decline in c-myc mRNA occurs which is followed by a cessation of cell growth and terminal differentiation. We transfected both cell lines with a plasmid containing the c-myc gene driven by the Molony LTR. All clones obtained from the MEL cell line expressed constitutive high levels of the transgene and were blocked in their ability to differentiate in response to chemical inducers. F9 derived clones expressed high levels of the exogenous c-myc gene, but the mRNA was down regulated in a similar fashion to the endogenous gene causing only a partial block to differentiation. To further support these findings we introduced, by stable transformation, into MEL cells a related myc family gene L-myc. A number of studies have shown greater than 90% sequence homology between C, N, and L-myc in several discrete areas of the gene. Although MEL cells do not express normally L-myc, all of the clones expressing high constitutive levels of the transfected gene fail to differentiate in response to the chemical inducer HMBA. Similar results were obtained recently in our lab with MEL/N-myc transfectants.

B. Identification of Regions in Human c-myc That are Involved in Cellular Differentiation. (In collaboration with W. Lee, University of Pennsylvania)

The involvement of c-myc in normal and neoplastic growth makes it important to understand its function(s) and the structural basis of some of its properties. Studies by Lee et al. have identified three areas that are essential for rat embryo cells cotransforming activity. The mapping of these areas was accom-

lished by the use of a large number of c-myc deletion/insertion mutants. We undertook a similar approach for identifying regions involved in differentiation.

#### C. The Role of the Jun Family of Genes in Differentiation of F9 Teratocarcinoma Cells. (In collaboration with J. Schuette and J. Minna)

We have analysed F9 teratocarcinoma cells for the expression of the different Jun family members and discovered that c-jun was not expressed in the stem cells, but low levels of expression were detected following induction with RA. Jun-B and Jun-D, on the other hand, were expressed at high levels in the uninduced and induced cells.

We then used these cells to characterize the transactivating activity of Jun-B. We also initiated experiments testing the transactivating activity of Jun-B on endogenous genes such as collagen type IV and laminin.

#### D. Identification of Genes Involved in Terminal Differentiation.

We have constructed a subtractive cDNA library from uninduced and induced MEL cells. We hope to be able to identify and clone genes which are differentially expressed in the induced cells and participate in the process of terminal differentiation.

#### Publications:

1. Kuehl WM, Bender TP, Stafford J, McClinton D, Segal S, Dmitrovsky E. Expression and function of the c-myc oncogene during hematopoietic differentiation. *Curr Topics Microbiol Immunol* 1988;141:318-323.
2. Segal S, Dmitrovsky E, De La Cadena M. The effect of a transfected c-myc protooncogene on cellular differentiation. *Mol Immunol* 1988;25:1129-1132.
3. Birrer JM, Raveh L, Dosaka H, Segal S. Transfected L-myc gene can substitute for c-myc in blocking murine erytroleukemia differentiation. *Mol Cell Biol* 1989;9:2734-2737
4. Schuette J, Nau M, Segal S, Viallet J, Minna JD. Protooncogene c-fos enhances transactivation by Jun-B, and transformation of primary rat embryo cells by Jun-B c-Jun and an activated c-Ha-ras gene. In preparation.

## —NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 07258-01 NMOB

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Etiology of Cutaneous T-cell Lymphomas

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Francine Foss, MD	Asst Prof Med NCI-USUHS	NCI-NMOB
	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	James Lynch, MD	Med Staff Fellow	NCI-NMOB
	Laura Markham, MS (1/3/89)	Res Assoc NCI-USUHS	NCI-NMOB
	Dat Nguyen, MD	Guest Researcher	NCI-NMOB

## COOPERATING UNITS (if any)

Robert Gallo, LTCB, DCE, NIH

## LAB/BRANCH

NCI-Navy Medical Oncology Branch

## SECTION

Molecular Biology of Differentiation

## INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD

## TOTAL MAN-YEARS:

4

## PROFESSIONAL:

4

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cutaneous T-cell lymphomas (Mycosis Fungoides and the Sezary Syndrome) comprise a group of indolent neoplasms of mature T-cell phenotype, the etiology of which is poorly understood. The clinical spectrum of these neoplasms varies from one of chronic skin involvement to one of aggressive disease with organ infiltration and circulating malignant T-cells. While in advanced stages, the malignant cells appear to be of the T-helper phenotype, it is unclear whether these cells originate from a T-cell event or whether the disease arises from events in an accessory cell which lead to the evolution of a clonal T-cell neoplasm. Previous studies suggest that early skin lesions may not demonstrate clonal T-cell proliferations. In addition, it has been suggested that retroviruses may play a role in the pathogenesis of Mycosis Fungoides, similar to the previously described role of HTLV-I in human acute T-cell leukemia/lymphoma, although there is currently no substantive evidence for this hypothesis. Our goal has been to study the biology and possible retroviral etiology of Mycosis Fungoides through studies using fresh tissues and cultured cells from patients. Ongoing clinical trials continue to provide tissues for analysis. Thus far, we have been able to establish six cell lines from bone marrow and lymph node sites from patients with Mycosis Fungoides, and two of these demonstrate possible retroviral reverse transcriptase activity.



## PROJECT DESCRIPTION

## Etiology of Cutaneous T-cell Lymphomas

PI:	Francine Foss, MD	Asst Prof Med NCI-USUHS	NCI-NMOB
	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	James Lynch, MD	Med Staff Fellow	NCI-NMOB
	Laura Markham, MS (1/3/89)	Res Assoc NCI-USUHS	NCI-NMOB
	Dat Nguyen, MD	Guest Researcher	NCI-NMOB

Collaborating Branches:

Robert Gallo, LTCB, DCE, NIH

Objectives:

1. To determine the origin of the malignant cells in Mycosis Fungoides by attempting to establish cell lines from various sites.
2. To study the biology of the Sezary cell lines with respect to growth factor production and response, oncogenic alterations, and sensitivity to chemotherapeutic and biologic agents.
3. To determine whether very early stage skin lesions represent polyclonal or monoclonal T-cell populations both for better understanding of disease pathogenesis and for possible use in diagnosis.
4. To define the possible role of retroviruses in the etiology of the disease.

Major Findings:Cell Culture Experiments

Previous efforts to establish long-term cultures of Sezary cells have yielded only one cell line, Hut 78. Immunophenotypic studies of this cell line and of and fresh Sezary cells from patients have shown that these cells represent a mature T-helper phenotype, expressing the CD4 antigen and lacking the TAC antigen, or IL2 receptor. The cells demonstrate a moderate but variable response to T-cell mitogens. Kinetic studies reveal that the cells in the circulating compartment are largely non-proliferating, in contrast to those in lymph node and skin. We have attempted to establish cell lines from blood, bone marrow and lymph node from patients with Mycosis Fungoides and have successfully maintained cells from bone marrow in four patients and from lymph node in two. These cell lines are of two types, one being characteristic of T-cells and one bearing markers of cells of monocytoid origin. Clones of these cell lines have been grown in serum-free media. Further characterization is underway.

### Retroviruses as Etiologic Agents

In collaboration with Dr. P. Browning and Dr. R. Gallo, we have been able to identify reverse transcriptase activity in cultured cells from two CTCL patients. Both of these lines represent a populations of cells which appear to be of monocytoid origin. Further isolation of this activity using density gradient centrifugation is underway. In addition, screening of DNA from CTCL cell lines by both PCR and Southern blotting has suggested the presence of novel retroviral sequences.

### Clonality of Early Stage Skin Lesions

We have developed techniques to evaluate small populations of clonal T-cells using PCR, in collaboration with I. Kirsch. We have evaluated lymph nodes at various stages of involvement to determine the level of sensitivity of the assay in isolating clonal populations in a largely polyclonal background. We have attempted to clone a segment of the clonal TCR-B rearrangement from a patient, and we hope to use this cloned DNA as a probe for in-situ hybridization studies of the skin.

### Proposed Course:

Several new techniques will be used to culture sezary cells, using a combination of mitogens and conditioned medias from the cell lines already established. We will attempt to immortalize cells in primary culture using amphotrophic retroviral constructs, in collaboration with M. Birrer. We hope to evolve a system by which cells can be maintained in-vitro for biologic studies and for drug and therapeutics testing.

We will pursue further characterization of the monocytoid cells currently maintained in culture. Genotypic and phenotypic analysis is underway. In vivo activity of these cells will be studies in nude mice. We will also attempt to grow similar cells from normal patients and from patients with other lymphoid malignancies.

We will continue to investigate the possible role of retroviruses in the etiology of this disease. Cell lines and fresh tissue supernatants will continue to be screened for presence of reverse transcriptase activity. DNA from involved tissues will be screened for retroviral-related sequences using PCR and Southern blotting. Retroviral isolation will be attempted if activity is found.

We will continue to evaluate early stage skin lesions for presence of clonal T-cell populations. Rearrangements in the TCR-gamma and delta loci will be defined. In situ hybridization techniques will be developed as outlined above. We hope that these investigations will yield a simplified screening technique which could be applied to early stage lesions as a diagnostic aid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06813-07 PB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Pediatric Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mark A. Israel	Head, Mol. Gene. Sec.	PB, NCI
Others:	P. Cohen	Biotechnology Fellow	PB, NCI
	C. Thiele	Senior Staff Fellow	PB, NCI
	L. Helman	Senior Staff Fellow	PB, NCI
	O. El-Badry	Guest Researcher	PB, NCI
	M. Cooper	Biotechnology Fellow	MB, NCI
	M. Lipinski	Guest Researcher	PB, NCI
	C. Gaetano	Guest Researcher	PB, NCI
	S. Plon	Biotechnology Fellow	MB, NCI

COOPERATING UNITS (if any) University of PA (A. Evans, J. Chatten: Johns Hopkins University (G. Hutchins); UCSF (M. Rosenblum); University of Upsalla (B. Westermarck); Rockefeller University (J. Friedman)

LAB/BRANCH

Pediatric Branch

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

8.5

PROFESSIONAL:

8.5

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have used human neuroblastoma as a model system to study the molecular events associated with the development of malignant tumors during childhood. Recent work has indicated that among histologically-indistinguishable solid tumors there are different genetic entities which correspond to cells at different stages in the differentiation of the tissue in which solid tumors arise. Such a schema has been hypothesized for both lymphoid and hematopoietic malignancies in which specific markers of differentiation have been extensively characterized, but tumors of solid tissues have previously been classified largely in terms of the organ in which they arise. Our current focus is to use recombinant DNA technology to identify and characterize tissue specific markers, to use these markers to develop clinically efficacious tumor nosologies, and to study the molecular mechanisms important for the regulation of tissue-specific differentiation and the arrest of growth which invariably accompanies it. Ongoing work in this area suggests that the molecular mechanisms that give rise to different tumor subgroups we have been able to identify are pathologic alterations in regulatory pathways that function during specific times in normal development.

During the past year we have expanded our studies in this area and sought profiles of gene expression that distinguish among tumors occurring at several different organ sites. In this manner we hope to subgroup a number of tumor types into biologically homogeneous groups. Ultimately, our goal is to use this information to better classify patients for therapeutic trials and to develop novel therapeutic approaches based on the specific alterations underlying the development of individual malignancies.

Accomplishments and Results:1. Evaluation of neuroblastoma cell differentiation:

We previously identified a series of genes highly expressed in neuronal cells and have utilized these to study the molecular mechanisms by which the in vitro growth arrest and differentiation of neuroblastoma tumor (NB) cell lines are mediated. We have utilized these genes as markers of neuroblastoma cell differentiation and determined that retinoic acid (RA) induces changes such occur during terminal neuronal cell differentiation. These changes include growth inhibition, neurite outgrowth, and high expression of neural associated genes such as GAP43 and neurofilament. cAMP treatment of neuroblastoma tumor cells does not markedly inhibit cell growth yet induces high level of expression of genes associated with a neuroendocrine phenotype, such as neuropeptide Y, pG2, an anonymous gene highly expressed in chromaffin cells, and insulin-like growth factor II (IGF-II). When both cAMP and RA are used to induce differentiation of NB cells, a neuronal morphology predominates. The expression of NPY, IGF-II, and chromogranin A persists in these cells, although pG2 expression decreases suggesting that commitment to a neuronal phenotype may turn-off expression of some neuroendocrine genes.

We have continued to evaluate the effect of NMYC expression on the growth and differentiation of NB cell lines. In ongoing experiments we have characterized a number of cDNA clones we previously isolated because their expression was dramatically altered by RA treatment. A gene whose expression was markedly decreased by RA was identified as ornithine decarboxylase, a key enzyme in the synthesis of putrescine, a polyamine required during DNA synthesis. A gene whose expression increases was identified as Na,K-ATPase, a protein highly expressed in mature neurons. We are also studying another gene, 37G1, that is transcriptionally regulated during RA induced differentiation. DNA sequence analysis of over 500 base pairs from each termini of this anonymous cDNA clone reveals no significant homology to sequences in GENBANK.

2. Evaluation of neuroendocrine differentiation

We have continued experiments to study the molecular events important for the regulation of neuroendocrine differentiation. Previously we cloned and characterized a series of cDNA clones that were highly expressed in mature neuroendocrine phenotype. Among these, pG2, an anonymous cDNA clone expressed uniquely in the adrenal gland, is of special interest since its expression marks a specific stage in adrenal medullary development that neuroblastoma tumors mimic. We have used our initial cDNA clone to isolate a full length cDNA clone of 1.6 kb and are currently determining the DNA sequence of this clone. In the course of experiments to characterize the *cis* regulatory elements of genes specifically expressed in neuroendocrine cells, we have determined more than 900 base pairs of DNA sequence 5' to the genomic site at which transcription of the gene encoding chromogranin A is initiated. These analyses have revealed the presence of two consensus sequences for cyclic AMP response elements at position -56 and a potential AP-2 binding site at position -380. In addition, we have also identified the presence of two 9 bp motifs present at positions -90 and -450 that are also present in the upstream regulatory regions of tyrosine hydroxylase and neuropeptide Y, two additional neuroendocrine lineage specific genes.

We have also examined the regulation of chromogranin A in functional assays evaluating the regulatory activity of DNA sequences located in the 2.5 kb of genomic DNA located upstream of the transcription

initiation site. In assays using CAT reporter gene constructs we have identified the promoter for this gene and we are currently examining whether any functional role for the various consensus sequences and conserved motifs we found can be identified. Experiments to identify cis-acting regulatory elements that may confer tissue specific gene expression are also underway using this approach.

In experiments designed to extend our laboratory work to the evaluation of current clinical problems, we have completed our analysis of neuropeptide Y expression in pheochromocytomas and demonstrated a statistically significant difference between the expression of this gene in benign tumors and its lack of expression in malignant pheochromocytomas. This may be of clinical importance since benign and malignant tumors cannot currently be distinguished from one another.

### 3. Expression of Class I HLA antigens in neuroblastoma

NB tumors and cell lines express low levels of class I MHC antigens that may contribute to the clinical course of this disease. We, therefore, sought to examine the regulation of Class I HLA gene expression in NB. Although we were unable to demonstrate a functional role for N-myc in the regulation of HLA expression, we did observe that NB tumor cell lines in which N-myc was not amplified expressed high levels of both HLA and beta-2 microglobulin. Interestingly, we found that in the adrenal medulla and embryonic tissue, the cells from which NB tumors are thought to arise, the expression of HLA was developmentally regulated and first detected late in the third trimester.

### 4. Lineage analysis of human neuroblastomas

In previous experiments, we found that NB cell lines and tumor specimens correspond to recognizable developmental stages of the maturing adrenal gland. We have extended these studies and evaluated, retrospectively, tumor specimens from neuroblastoma patients to determine whether our characterization of the developmental features of a specific tumor may be of prognostic importance. In other experiments we are attempting to identify factors that regulate the proliferation and differentiation of fetal adrenal neuroblasts during normal development. We will then determine whether such factors could modify the malignant behavior of neuroblastoma tumor cell lines.

### 5. The role of growth factors in the proliferation of neuroblastoma tumor cells

We have extended our study of the role of IGF-II in the proliferation of neuroblastoma cell lines and tumors. We have demonstrated that SK-N-AS, a neuroblastoma cell line, synthesizes, processes, and secretes biologically active IGF-II and contains on its surface type I IGF receptors that can be stimulated by IGF-II to induce SK-N-AS proliferation. Interestingly, IGF-II can only be detected in the adult adrenal medulla, and we have found its expression only in pheochromocytoma, a tumor of mature chromaffin tissue and in NB cell lines we previously identified as corresponding to the latest recognizable stage in chromaffin cell development. In other experiments we have found that most neuroblastoma cell lines that do not produce IGF-II will, nonetheless, proliferate in response to its addition to culture media suggesting a role for this factor in the growth of these tumor cell lines. In concert with these findings, we have detected IGF-II production by stromal tissues in some neuroblastomas, and are pursuing the possible role of IGF-II in mediating the paracrine stimulation of malignant cell growth in these tumors.

## 6. Evaluation of astrocytic differentiation

We are currently pursuing a line of investigation that we hope will provide insight into the molecular mechanisms that mediate glial cell differentiation. Previously we studied the expression of a number of different proto-oncogenes in glioma tumor cell lines and determined that the expression of c-sis might be a marker of tumors that correspond to cells that are found rather late in the maturation of astrocytes. In recent studies we have cloned and determined the DNA sequence of a cDNA clone that encodes human glial fibrillary acidic protein (GFAP). GFAP is currently the only known marker of the astrocytic lineage and in ongoing experiments we have cloned the genomic region located 5' to these coding sequences. We are evaluating this region in reporter gene constructs to characterize the genetic elements important for the tissue specific expression of this gene. We anticipate that these experiments will provide a basis upon which to pursue an examination of why genes marking the fully-mature phenotype of tissues in which tumors arise are not typically expressed in most tumors arising in those tissues.

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## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06830-19 PB

## PERIOD COVERED

October 1, 1988 - September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Infectious Complications of Malignancy and HIV Infection in Children

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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## COOPERATING UNITS (if any)

Medicine Branch, Surgery Branch, NCI; Diagnostic Microbiology, Department of Transfusion  
Medicine, CC; Bethesda Naval Hospital; Duke University; Medical Illness Counseling Center

## LAB/BRANCH

Pediatric Branch

## SECTION

Infectious Disease

## INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

5.0

## PROFESSIONAL:

4.0

## OTHER

3.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies are devoted to developing methods to define cancer patients who are at risk for developing serious infection, to improving the ability to diagnose these infections early, to treat them effectively, and ultimately to prevent them. We are developing new therapeutic approaches based on the ability of new antibiotics particularly the beta-lactams and the quinolones. We have shown that certain beta-lactams used as single agents can replace the need for combination antibiotic therapy. Our studies are also defining the appropriate antibiotic therapy for documented infections, particularly the role of oral antibiotic therapy; the necessary duration of empiric therapy for patients with unexplained fevers and the choice of empiric antifungal therapy.

We have developed a unique model for studying the pathophysiology, natural history, treatment and prevention of invasive candidiasis in the neutropenic host. This model permits the testing of new antifungal agents as well as immunoregulatory agents. To prevent infections we are evaluating the role of passive immunization with a pooled immunoglobulin preparation that contains activity against the enterobacteriaceae as well as the pseudomonads. We are also studying other immunoregulatory agents that may serve as adjuncts to the treatment of infection, including interleukin 1 and 2, GM-CSF and M-CSF.

We have developed a program to evaluate the benefits of antiretroviral agents in children with HIV infection. To date, these have focused on studies with dideoxynucleosides. Studies with immunoregulatory agents and with biologicals (e.g., rCD4) are also underway.



Professional Personnel (Continued):

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Accomplishments and ResultsA. Diagnosis, Management and Prevention of Infectious Complications in Cancer Patients

1. To determine the role of new beta-lactam antibiotics in providing simpler, safer and effective therapy for neutropenic cancer patients who become febrile, we have conducted a randomized trial comparing a third-generation cephalosporin (ceftazidime) to a carbapenem (imipenem-cilastatin) for initial empirical therapy. The goal of this study is to both evaluate the role of these agents in providing safe initial therapy as well as determining whether the numbers of modifications of the primary antibiotic varies in patients with defined infection or prolonged granulocytopenia. From March, 1986 through March, 1989, we enrolled 337 evaluation episodes of fever and neutropenia, randomizing these to initial ceftazidime (170 episodes) or imipenem. Both regimens provided comparable primary therapy. More modifications of the initial regimen were necessary for patients with documented infection who were randomized to ceftazidime, and there were more second infections in this group. These were primarily with gram-positive bacteria. However, there were no differences in infection related morbidity or mortality. On the other hand, there were more complications with imipenem, including higher incidence of *C. difficile* diarrhea and a higher degree of intolerance due to nausea and vomiting. Overall, both antibiotics appear useful, have different strengths and weaknesses and confirm that various alternatives can be employed to provide safe monotherapy for the majority of febrile neutropenic cancer patients.
2. In a randomized trial, we have demonstrated that it is appropriate to continue empiric antibiotic therapy for a limited (i.e., 2 week) course for patients who have defervesced following the initiation of antibiotics but who remain febrile.

In a follow-up study, we are comparing the use of a new class of oral antibiotics, the quinolones, for patients who have defervesced on parenteral therapy, have no defined site of infection, and remain persistently granulocytopenic. This study has considerable importance, since it can serve to re-define the role of inpatient versus outpatient therapy for treating infectious complications that occur in tandem with cancer therapy. To date, 56 patients have been randomized.

3. To decrease the frequency of infectious complications associated with indwelling intravenous catheters of the Hickman-Broviac type, we have performed a randomized trial to compare the Hickman-type catheter to a subcutaneously implanted catheter (Port-a-Cath). Since the subcutaneous catheter requires less manipulation, our hypothesis is that it will have a low incidence of infection. However, if infected, it is possible that these infections will not be

capable of eradication unless the catheter is removed. One-hundred patients were randomized, 48 to a Hickman catheter and 52 to a Port-a-Cath. Overall, 10,592 days of catheter placement were evaluated in the Hickman group and 14,634 catheter days for the Port-a-Cath group. Analysis to date shows no difference in the frequency of infectious or noninfectious complications.

4. We reviewed pre-antibiotic culture data from 550 patient episodes of fever and neutropenia, comparing those episodes in which patients had indwelling I.V. catheters, to those in which they did not. Results of this analysis have helped determine the appropriate initial antibiotic management of febrile neutropenic patients with catheters. There were more gram-positive bacteremias in those patients with catheters vs. those without, but when individual patterns of infection were analyzed (i.e., specific organisms), no significant differences in these populations were seen. The data underscore the need for a heightened awareness of certain potential bacteremias in patient with catheters, but do not support the routine inclusion of vancomycin in these patients.
5. In order to help predict which patients will eventually require modifications of their empirical antibiotics, we analyzed initial characteristics of febrile neutropenic patients receiving different empirical regimens (single agent and combination therapy). Certain characteristics were more often associated with the eventual need for antibiotic modifications, although both regimens were equally effective. Knowledge of these characteristics predictive of the need for modification may be helpful in the management of persistently neutropenic patients.
6. To reduce the incidence of infection in patients who have protracted neutropenia, we evaluated, in a double-blind randomized trial, the value of passive immunization with a pooled intravenous immunoglobulin (IVIG). We also assessed the utility of this immunoglobulin in attenuating the types of infections which occur. Of 65 evaluable episodes, 33 were randomized to IVIG and 32 to placebo. No benefit from the IVIG was observed.
7. Because patients receiving IL2 have an increased incidence and severity of certain bacterial infections, the functional activity of PMN from patients receiving IL2 was assessed. A significant impairment in chemotaxis and Fc receptor expression was found. Studies are underway designed to elucidate the mechanism of PMN dysfunction in these patients. It does not appear that IL2 alone accounts for the observed changes. IL2 may induce production of other cytokines that, in turn, adversely affect PMN function. Currently, we are assessing the effects of IL6 *in vitro*.
8. In preparation for *in vivo* administration of elutriated monocytes to patients with progressive infection, a variety of studies are underway. We have demonstrated that these cells have good phagocytic, chemotactic and microbicidal activity. We are studying their production of cytokines such as IL1, TNF and GM-CSF, in order to obviate potential toxicities and maximize efficacy. Optimal culture and storage conditions are being determined, and potential for *in vitro* activation of these cells explored.

Preliminary data has been obtained from a patient with aplastic anemia and a rapidly progressive aspergillus infection. The patient was given nine transfusions with elutriated monocytes. Preliminary results indicate little toxicity, trafficking of the cells to the site of infection, and stabilization of the infectious process.

9. In an attempt to reduce the duration of neutropenia associated with cytotoxic chemotherapy, we have initiated a prospective randomized trial in children with sarcomas whereby,

following chemotherapy, they are randomized to receive or not receive rGM-CSF. The goal of this study is to determine whether the cytokine will reduce the incidence and severity of the fever of infection usually associated with neutropenia. Should this prove effective, it may permit altering chemotherapy schedules in a manner that might better optimize their anti-tumor efficacy.

10. We have demonstrated that potential importance of non-aeruginosa *Pseudomonas* spp. infections in cancer patients. We have reviewed the characteristics of infections due to these organisms, and based on this experience, have outlined guidelines for management.

#### B. Experimental Mycoses: Preclinical and Clinical Studies

Invasive fungal infections are significant and increasing problems of morbidity and mortality in cancer patients and those with AIDS. Accordingly, we investigated the antifungal activity, pharmacokinetics, and immunomodulatory properties of several of these most promising agents for potential use in our high risk patient populations.

1. We developed a system consisting of three animal models of experimental disseminated candidiasis for targeting antifungal chemotherapy to three specific clinical patterns: acute, subacute, and chronic disseminated candidiasis; and for three antifungal regimens: preventive, early, and delayed treatment.
2. We demonstrated that three potent antifungal triazole compounds (itraconazole, fluconazole, and SCH-39304) were most effective when administered as preventive or early antifungal chemotherapy and have the clinical potential for use in early empirical antifungal therapy.
3. We demonstrated that the new antifungal triazoles (itraconazole, fluconazole, and SCH-39304) were as effective as amphotericin B plus flucytosine in early treatment of experimental disseminated candidiasis but that amphotericin B plus flucytosine was more effective against chronic (hepatosplenic) candidiasis.
4. These experimental antifungal studies provided the scientific rationale for design of a multicenter clinical to test the concept of early empirical antifungal therapy with fluconazole and for the first phase I trial of a systemic antifungal agent (fluconazole) in children. This study will commence shortly.
5. We have demonstrated the superior efficacy of cilofungin (LY-121019) when administered by continuous infusion compared to intermittent infusion against disseminated candidiasis in persistently granulocytopenic rabbits, representing for the first time an experimental rationale for continuous infusion of a systemic antifungal compound.
6. We identified the microbiological basis for amphotericin B resistance in *Trichosporon beigeli* (an emerging and often fatal systemic fungal pathogen in cancer patients). We found *T. beigeli* was inhibited but not killed by amphotericin B at safely achievable plasma concentrations. The minimum fungicidal concentration was markedly greater than the minimum inhibitory concentrations, thus suggesting the need for other antifungal compounds to treat this infection in neutropenic patients.
7. We have shown using *in vitro* timed kill assays that cilofungin is a highly fungicidal compound against *Candida albicans*, that combinations with other antifungal compounds do not

appreciably augment its activity, and that the compound is fungicidal against *Torulopsis glabrata* at higher concentrations.

8. We demonstrated that cilofungin (LY-121019) is excreted via the biliary tract, has a short plasma half-life following first order kinetics with single dose administration but with continuous or frequent intermittent infusion, we demonstrated the non-linear saturable pharmacokinetics of cilofungin (LY-121019), thus accounting for the heretofore unexplained basis of accumulation of this promising compound in human volunteers.
9. We have demonstrated that fluconazole and SCH-39304 have long plasma half-lives and extensive tissue penetration into multiple tissue sites, including the CSF, brain, choroid, and vitreous fluid, thus permitting the administration of these compounds to patients with CNS fungal infections.
10. We demonstrated that a new antifungal triazole (BAYR-3783) is converted into active metabolites, one of which has an exceedingly long plasma half with penetration into the central nervous system.
11. We demonstrated that *Candida* cholecystitis, an increasingly reported manifestation of invasive candidiasis, may be effectively treated by drainage and IV amphotericin B and that the biliary concentrations of amphotericin B exceed those of plasma by two to eight-fold.
12. We studied the effects of amphotericin B, 5-fluorocytosine, ketoconazole, fluconazole, cilofungin, and SCH-39304 on the granulocyte function and found that within the usual therapeutic concentrations the new and established antifungal compounds either did not affect or enhanced chemotaxis, phagocytosis, intracellular killing, and superoxide generation.
13. We developed a new method of studying continuous infusion pharmacokinetics in rabbits by utilizing a double silastic central venous catheter technique and a portable minipump, permitting the study of the pharmacokinetics of continuous infusion of antimicrobial compounds as well as systemic immunomodulators.
14. In collaboration with the Antifungal Therapy Committee of the Medical Society of the Americas, we documented a substantial increase in the usage of systemic antifungal agents worldwide and within the the NIH Clinical Center, consistent with the increased recognition and frequency of invasive mycoses in compromised patients.
15. We demonstrated that the depth, duration and recovery from granulocytopenia are important determinants in the clearance of experimental disseminated candidiasis; we further showed that recovery from granulocytopenia is not a sufficient condition for clearance of tissue candidiasis if profound granulocytopenia was present during the time of infection.
16. These studies serve as the foundations for developing a rational approach to the use of G-CSF for the prevention and treatment of disseminated candidiasis. Laboratory investigations with this compound will commence during the summer 1989.
17. We demonstrated that two synthetic immunomodulators being considered for introduction into the United States for clinical investigation for enhancing granulopoiesis (a muramyl dipeptide derivative and a pteridine derivative) had little activity in promoting recovery from granulocytopenia in granulocytopenic rabbits.

18. Germination from the yeast phase to the hyphal phase is a key step in the pathogenesis of tissue invasion for *Trichosporon beigelii* and *Candida albicans*. We identified the individual amino acid, carbohydrate, pH, and temperature signals that determine this initial step of invasion.
19. We identified a previously unrecognized phenomenon of stable and unstable phenotypic conversion of *T. beigelii*, which has implications for microbiological diagnosis and for pathogenesis. We further characterized the biochemical basis for this conversion as being inducible by membrane phospholipids and cholesterol but not by triacylglycerols, proteins, carbohydrates, or nucleic acids.
20. We demonstrated that *Candida lipolytica* is a relatively a virulent pathogen that may be the source of sustained fungemia from an intravascular focus.
21. We have characterized the factors influencing the expression of antigenemia due to an immunodominant 48 kD cytoplasmic antigen of *Candida albicans* in experimental disseminated candidiasis.
22. We have demonstrated the potential utility of an immunoassay detecting the 48 kD immunodominant cytoplasmic antigen for diagnosis of disseminated candidiasis cancer patients in a multi-center trial.

#### C. HIV Infection in Children: Clinical and Preclinical Studies

1. We have continued our Phase I-II studies of children with symptomatic HIV infection. Since beginning this project in December, 1986, we have evaluated approximately 110 children, enrolling the majority into clinical trials.
2. Our initial study of AZT, administered either by continuous intravenous infusion or on an intermittent schedule, are completed. Both routes of therapy appeared to offer benefit, particularly for children with neurodevelopmental deficits. However, the extent of this benefit, appears to be greater for children treated by the continuous intravenous schedule. To validate this, we will soon begin a randomized study comparing AZT administered on a schedule that maintains steady-state kinetics in the plasma and CSF to one in which the drug is delivered on an intermittent schedule.
3. Our prior studies with AZT demonstrated that the dose-limiting toxicity was myelosuppression related to both dosage and duration. Consequently, we have evaluated two schedules to spare AZT-induced myelosuppression. The first alternates AZT with ddC, and the second combines it with rH-GM-CSF. In the study with ddC, we first evaluated this newer dideoxynucleoside in a limited phase I trial, studying four dosage levels (0.015, 0.020, 0.030, and 0.040 mg/kg/q/6h) administered over a 8-week period. Fifteen patients were treated. We observed decreases in P24 antigen in 5/9, increments in CD4 counts in 8/15 during the 8-week trial of ddC as a single agent. We also treated 13 of these 15 patients with an alternating schedule of ddC and AZT and found this to be non-toxic and tolerable during a minimum follow-up period of 6 months.
4. We also initiated a protocol to evaluate the combination of AZT with colony stimulating factor in order to overcome the myelosuppression of AZT. To date, one patient has been treated on this protocol.

5. In a search for effective, less toxic regimens, we initiated a Phase I-II trial of dideoxyinosine (ddI) in children in January, 1989. To date 27 children have been enrolled at several dosage levels (20, 40, 60, 120 and 180 mg/m<sup>2</sup>/every 8 hours). This protocol enrolls both children who have received no prior anti-retroviral therapy as well as children who have become refractory or intolerant to AZT. To date, 27 children have been enrolled. The first four dose levels are completed in the 5th level (180 mg/m<sup>2</sup>/day) is accruing. No significant toxicity has been observed and both objective and subjective responses have been seen (albeit still preliminary) in both previously untreated and refractory/intolerant children.
6. We have demonstrated that *B. pertussis* can result in serious pulmonary infection in HIV-infected children and should be considered in the differential diagnosis of pneumonic processes.
7. PMN from HIV-infected children were demonstrated to have significant impairment in their ability to phagocytose and kill *S. aureus*. *In vitro* incubation with GM-CSF corrected the bactericidal defect. These findings may help explain the increased incidence of *S. aureus* infection in this population, and suggest a potential therapeutic role for GM-CSF. Similar studies assessing G-CSF are ongoing.
8. We are assessing the potential role of HIV antigens (e.g., GP 41 and GP 120) on the modification of functional activity of normal PMN. Preliminary data indicates that certain components may have a significant effect on important functional parameters, which may in part explain certain clinical observations.
9. We have studied the effects of antiretroviral agents on PMN function *in vitro*. AZT, and ddC had no demonstrable effects on PMN functional activity. Preliminary data indicate that ddI may actually enhance bactericidal activity of PMN *in vitro*.

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## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06840-14 PB

## PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Treatment of Acute Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

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## COOPERATING UNITS (if any)

Medicine Branch, NCI (A. Fojo, K. Cowan, L. Neckers); Navy, NCI (L. Kirsch); Children's Cancer Study Group (G. Reaman).

## LAB/BRANCH

Pediatric Branch

## SECTION

Leukemia Biology Section

## INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

5.0

## PROFESSIONAL

3.0

## OTHER

2.0

## CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical research into the biology and treatment of acute leukemia is pursued with particular emphasis on acute lymphoblastic leukemia (ALL) of childhood. Major issues being addressed include: 1) development of therapeutic strategies aimed at improving overall prognosis of children with ALL, 2) investigation into the mechanisms of treatment failure with particular emphasis on evaluation of pharmacologic approaches to leukemic therapy, 3) characterization of adverse sequelae of antileukemic therapy and design of treatment regimens which avoid them, and 4) studies of the biology of ALL aimed at improving our basic understanding of the biology of this disease, identifying new diagnostic and prognostic tests and providing insight into the biologic basis for treatment failure.

An earlier ALL treatment protocol demonstrated that high-dose, protracted systemic methotrexate infusions could substitute for cranial radiation as central nervous system (CNS) preventive therapy for the majority of patients with ALL. Analysis of data from this study also identified a patient group at particular risk for CNS relapse. A new, high risk protocol has been devised in an attempt to improve the prognosis for these and other poor risk patients. The results to date indicate that this therapy is highly effective in preventing both systemic and central nervous system relapses while avoiding the use of cranial radiation. In patients in the average risk category, a comparison of two forms of CNS preventive therapy (intrathecal vs high dose methotrexate) is under way. A major, multi-institutional pharmacologic monitoring protocol is in progress which is studying the relationship between the bioavailability of orally administered maintenance chemotherapy and relapse in children with ALL. On the basis of in vitro studies indicating that Interleukin-2 will induce phenotypic and functional maturation in acute lymphoblastic leukemia cells, a Phase I study of this potential antileukemic approach has been pursued. A study demonstrating expression of the  $\beta$  II-2 receptor on hematopoietic malignant cells offers a potential avenue for future therapy. Detailed analysis of the immunologic and molecular phenotype of acute lymphoblastic leukemia has led to the concept of a hierarchy of differentiation for both T cell and pre-B cell ALL. Studies are in progress to determine the relationship of molecular phenotype to prognosis.

## Objectives

1. To develop effective treatment strategies which will improve the event-free survival of children with ALL, with particular emphasis on a) the development of alternative CNS preventive therapy and b) improvement of treatment for patients with poor risk features.
2. To characterize the long-term adverse sequelae of antileukemic therapy and design treatment regimens that avoid them.
3. To study the clinical pharmacology of antileukemic agents with the objective of optimizing ALL treatment through: a) exploration of the pharmacologic basis of treatment failure in ALL, b) development of new treatment strategies, with currently available antileukemic agents, which are based on sound pharmacologic rationale, and c) identification of promising new antileukemic agents.
4. To conduct studies of the biology of ALL in an attempt to increase our basic understanding of this disease and to identify biological characteristics which will provide avenues for new therapeutic approaches.

## Methods and Major Findings:

### A. Treatment Studies of Acute Lymphoblastic Leukemia

#### 1. NCI 77/02/CCG 191 Treatment Protocol

A randomized protocol investigating the efficacy of high dose intravenous methotrexate infusions as CNS preventive therapy. Patients received either cranial radiation plus intrathecal methotrexate or high dose 24-hour intravenous methotrexate infusions. One-hundred-eighty-one (181) average and high risk patients were randomized on this study. The overall remission rate was 98%. The continuous complete remission rate is approximately 67% at three years for the entire study group. With a median duration on study of 82 months, there is no significant difference in the CNS relapse rate for either treatment group. Longitudinal evaluation of neuropsychological function has demonstrated a striking decrease in IQ test scores and impaired academic achievement in children treated with cranial radiation and intrathecal chemotherapy. No such changes have been observed in children treated with high dose methotrexate. The results of this study not only demonstrated that alternative CNS preventive therapy is feasible and as efficacious as cranial radiation and IT MTX, but also served to focus attention on the importance of avoiding neurotoxic regimens using cranial radiation. This study led to the development of our two current clinical trials discussed below.

#### 2. NCI 83-P/CCG 134P

The major aim of this pilot protocol is to demonstrate that high risk patients can be effectively treated on a regimen that uses CNS preventive therapy devoid of cranial radiation. To date, 102 patients have been entered on study; 96% achieved complete remission. With a median duration on study of 39 months, the event

free survival (at 24 months) is approximately 70%. The occurrence of isolated CNS relapse in only two patients, to date, suggests that effective CNS preventive therapy can be achieved without the use of cranial radiation in high risk patients.

### 3. NCI 84-A/CCG 144

This protocol randomizes average risk patients in one of two forms of CNS preventive therapy - either high dose methotrexate infusions or intrathecal methotrexate alone. One hundred sixty-six patients have been randomized on study. With a median potential duration on study of 26 months, there is no significant difference in the CNS or bone marrow relapse rate in either treatment arm. Although these results suggest that intrathecal MTX is as effective for CNS preventive therapy as HDMTX infusions for average risk patients, further follow-up is necessary before this statement can be made definitively.

## B. Pharmacologic Approaches to Leukemic Therapy: Relationship to Treatment Failure

A detailed study of the bioavailability of the major orally administered antileukemic agents is being undertaken in an attempt to examine the reasons for treatment failure in children with ALL.

### 1. Prospective Evaluation of Oral 6-MP and MTX Bioavailability.

This study is attempting to correlate the results of prospective periodic pharmacokinetic bioavailability studies of 6-MP and methotrexate with relapse rate and remission duration in a multi-institutional setting. Approximately 90 patients have been entered to date. The bioavailability and pharmacokinetics of oral 6-MP and MTX are studied on four separate occasions during the course of maintenance therapy in children with average and good risk ALL. Erythrocytes are periodically examined for MTX and 6-MP nucleotide content. To date, clinical information regarding disease status and toxicity in this group is still too incomplete for meaningful analysis. However, we have begun to analyze the "population" pharmacokinetics of these two agents. We have confirmed the wide inter-patient variability in plasma MTX and 6-MP concentrations following oral administration under standardized conditions, and have defined the "normal" range of the area under the plasma concentration-time curve (AUC) for both drugs. We are also able to evaluate the intra-patient variability in drug bioavailability; preliminary analysis reveals much greater variability with 6-MP than with MTX. This variability within the same patient may limit the application of therapeutic drug monitoring of 6-MP therapy. The absorption of these two agents does not appear to decline over the course of maintenance therapy, and the degree of absorption of one agent does not correlate with how well or poorly the other drug is absorbed. When patient accrual is complete and sufficient follow-up is available, the final pharmacokinetic analysis and clinical correlations will be made.

### 2. Factors Affecting the Bioavailability of 6-MP and MTX.

In other studies, we are examining several specific factors which may impact on the bioavailability of 6-MP and MTX in patients:

MTX-6-MP Interaction.

We recently discovered a pharmacokinetic interaction between MTX and 6-MP. The absorption of 6-MP was found to be 20% higher when the 6-MP was co-administered with MTX than when 6-MP was administered alone. These findings are believed to be the result of MTX inhibition of the first pass metabolism of 6-MP, the process that limits 6-MP absorption. The clinical significance of this finding, at routine doses of 6-MP and MTX, is unclear.

Chronopharmacology of Maintenance Therapy.

A retrospective study recently cited a five-fold greater risk of relapse in children with ALL who took their oral maintenance therapy on a morning rather than an evening schedule. A possible explanation was that the difference in the efficacy of the morning and evening schedules reflected a difference in total drug exposure that resulted from circadian periodicity in the disposition of 6-MP and MTX. We have investigated the chronopharmacokinetics of both 6-MP and MTX in children with ALL to determine if there is a pharmacokinetic basis for this observation. Children were fasted and given either 6-MP or MTX at both 8:00 AM and 8:00 PM. No significant differences were noted in the plasma concentrations of either 6-MP or MTX on the two administration schedules. Thus, we are unable to confirm that diurnal variation in the absorption or elimination of 6-MP and MTX plays a role in the response to maintenance therapy with these drugs.

Saturation of 6-MP Pre-Systemic Metabolism.

6-MP bioavailability is limited primarily by metabolism to the inactive metabolite, thiouric acid (TU). Based on evidence of saturation of first pass metabolism in monkeys it appeared that with a substantial increase in the dose of 6-MP, xanthine oxidase, the catabolic enzyme that converts 6-MP to TU, might be saturated leading to an increase in the fraction of the dose absorbed. We tested this hypothesis in children with ALL and found that the extent of absorption actually decreased slightly when the dose was increased from 75 to 500 mg/m<sup>2</sup>. However, at the 500 mg/m<sup>2</sup> dose level all patients achieved cytotoxic peak 6-MP concentrations (1 to 10 mmol/L). It appears that the use of these higher dose levels is not associated with the risk of unexpectedly high drug concentrations resulting from saturation of presystemic metabolism.

3. Alternate Dosing Methods.

We have investigated alternate routes of administration for both 6-MP and MTX as possible methods of optimizing maintenance chemotherapy with these agents.

Subcutaneous MTX.

We have studied subcutaneous MTX as a parenteral alternative to oral administration. The subcutaneous route has several potential advantages

including slow release of the drug leading to more prolonged drug exposure, ease of administration, and more complete and less variable absorption. Two dose levels (7.5 and 40 mg/m<sup>2</sup>) were studied, and each child was monitored twice after an oral dose and after the same dose administered subcutaneously. The subcutaneous dose was well tolerated and well absorbed at both dose levels studied. In contrast, the oral dose produced comparable plasma concentrations at the lower dose, but total drug exposure (AUC) at the higher dose was only one third that achieved with the subcutaneous dose, presumably a result of saturation of the mechanism responsible for MTX absorption in the gastrointestinal tract. Subcutaneous administration appears to be a viable alternative in patients with poor gastrointestinal absorption at lower doses and in patients receiving doses greater than 30 mg/m<sup>2</sup>.

#### 4. Alternative Maintenance Agents.

We are actively studying nonclassical antifolates which may be alternatives to MTX, such as trimetrexate and piritrexim. A phase I trial of trimetrexate on a once weekly for 3 weeks schedule has recently been completed and a phase I trial of oral piritrexim is ongoing (see *Clinical Pharmacology Project Report*).

#### C. Drug Resistance.

In order to overcome drug resistance we must also define the mechanisms through which leukemic cells become resistant to the antileukemic agents. In collaboration with laboratories in the Medicine Branch we have screened lymphoblasts from our patients on a molecular level for the presence of multidrug resistance caused by overexpression of *mdr-1/P-170* and glutathione S-transferase (GST). In addition, mechanisms specific to individual agents, such as the overexpression of dihydrofolate reductase (DHFR), the target enzyme of MTX have also been evaluated in leukemic cells.

##### Multi-Drug Resistance.

Lymphoblasts from 28 patients were studied for evidence of *mdr-1/P-170*, the gene encoding for the plasma membrane glycoprotein associated with multidrug resistance, using RNase protection, RNA *in situ* hybridization and immuno-histochemistry. Overexpression without gene amplification was identified in the cells of three relapsed patients and from one patient at diagnosis (this patient failed to achieve a complete remission with induction therapy). *In situ* hybridization, immuno-histochemistry, and drug uptake studies demonstrate that this overexpression is heterogeneous. It appears from these studies that overexpression of *mdr-1/P-170* is one mechanism of drug resistance in ALL.

GST is a drug metabolizing enzyme that is overexpressed in a multidrug resistant breast cancer cell line (MCF-7). Expression of GST is being evaluated in lymphoblasts from our patients. Thus far, higher levels of expression have been found in the cells from 2 relapsed patients than in the cells from three patients obtained at diagnosis, suggesting that GST may also be a marker of acquired resistance in ALL. A technique to assess

GST expression, which involves *in situ* hybridization, is currently being used to evaluate a larger group of patients to determine the overall incidence of this phenomenon.

#### Specific Mechanisms of Drug Resistance.

DHFR gene amplification is one of the most studied and best understood forms of resistance *in vitro*. However, we have been unable to document either overexpression (n=8) or amplification (n=11) of the DHFR gene in lymphoblasts from our patients. Other mechanisms of resistance to MTX may be of more importance and should be evaluated.

#### D. Molecular Biology of Acute Lymphoblastic Leukemia

Collaborative studies are investigating the status of immunoglobulin gene rearrangement and T-cell receptor gene status in acute leukemic lymphoblasts. Studies to date have enabled us to construct a hierarchy of differentiation for both pre-B cell precursor ALL (by immunoglobulin gene rearrangement). Recent and for T-cell rearrangement (using T-cell receptor gene rearrangement). Recent studies, performed in collaboration with the NCI/Navy Medical Oncology Branch, have been aimed at determining whether there is a correlation between molecular genotype in ALL and a variety of biologic and clinical features known to have a prognostic import (e.g. cytogenetics, initial white blood cell count, FAB morphologic classification, etc.) as well as with treatment outcome. Lymphoblasts obtained at diagnosis from patients treated on our "front line" ALL treatment protocols are prospectively studied with cytogenetics, immunophenotyping (using FACS analysis and a panel of monoclonal antibodies), and molecular characterization. An analysis of this data is in progress to determine whether biologically and/or clinically significant correlations exist.

#### E. Interleukin-2 and Acute Lymphoblastic Leukemia

##### IL-2 as therapy for Acute Lymphoblastic Leukemia

*In vitro* studies with IL-2 have demonstrated its ability to induce phenotypic and functional maturation in a subset of acute leukemic lymphoblasts. This observation led to the development of a Phase I trial in patients with hematologic malignancies which is currently in progress. IL-2 is administered intravenously by continuous infusion for five days on a weekly, for three successive week, schedule. The starting dose of  $10^5$  units/m<sup>2</sup>/day has been escalated according to standard phase I guidelines. While the aim of this ongoing study is to determine the maximally tolerated dose of IL-2 on this continuous infusion schedule, biological studies are also being performed to evaluate the effects of IL-2 *in vivo*. 25 date, 17 patients have been entered on this study. Once the maximally tolerated dose on this schedule is defined, we plan to pursue a phase II study of this approach in patients with ALL to determine whether the provocative *in vitro* findings of IL-2 induced maturation and killing of T-cell lymphoblasts can be induced in patients with ALL.

### The $\beta$ Subunit of the IL-2 Receptor.

The receptor for interleukin-2 consists of two subunits,  $\alpha$  (P55), recognized by the monoclonal antibody anti-TAC and  $\beta$  (P70/75) to which there yet is no monoclonal or polyclonal antibody. Although the distribution of the  $\alpha$  subunit of hematopoietic neoplasms has been well characterized, little was known about  $\beta$  subunit expression. In collaboration with the Medicine Branch we have demonstrated that whereas both lymphoid and non-lymphoid acute leukemias are for the most part lacking in  $\alpha$  subunit expression, they consistently express the  $\beta$  subunit of the IL-2 receptor. These findings suggest that the  $\beta$  subunit of the IL-2 receptor might be a useful marker for hematopoietic malignancies and may also represent a possible target for immunotherapy.

## F. New Agent Studies in Relapsed Patients

### 1. Phase I and Phase II Trials.

The major focus of our studies for relapsed patients with ALL is on phase I and phase II trials of investigational agents. Emphasis is placed on those new drugs, examined in our laboratory, for which there exists a significant pharmacologic rationale for their use in the treatment of leukemia. Within the past year we have carried out and completed three phase I trials, including fazarabine, piritrexim and Interleukin-2. For a detailed listing and discussion of these new agent studies the reader is referred to the *Clinical Pharmacology Project Report*.

### 2. New Intrathecal Agents.

In recent years, we have focused attention on the development of new pharmacologic approaches to the treatment of CNS leukemia. Although numerous drugs are available for systemic administration to treat ALL, the number of agents suitable for intrathecal use is limited; no new intrathecal agents have been identified in over 25 years. In contrast to the successful treatment of systemic leukemia which is predicated on the use of combination chemotherapy, the extremely limited number of intrathecal agents restricts clinicians to the use of only one or two agents (e.g. MTX and Ara-C) which belong to the same drug class (antimetabolites). It is conceivable that if effective new intrathecal agents could be identified the development of combination intrathecal chemotherapy regimens could have the same impact on the control of CNS leukemia as combination chemotherapy has had on control of bone marrow disease. In addition, since CNS preventive therapy with cranial radiation is associated with adverse CNS sequelae, new intrathecal agents are also needed for CNS preventive therapy. Thus, the identification of effective new intrathecal agents has become an appropriate and important priority. Two new intrathecal agents developed in our nonhuman primate model are currently undergoing clinical study, intrathecal diaziquone (AZQ) and intrathecal 6-mercaptopurine. These studies are detailed in the *Clinical Pharmacology Project Report*.

# G. Prospective Study of Neurotoxicity of CNS Preventive Therapy

More recently, our clinical studies of adverse sequelae have focused on the long-term follow-up of patients treated on the 77-02/191 protocol in which patients were randomized to receive either cranial radiation plus intrathecal chemotherapy or HDMTX as CNS preventive therapy. A major objective of this study is to prospectively compare the incidence of adverse CNS sequelae with the two forms of CNS preventive therapy. We are longitudinally evaluating patients with a comprehensive battery of age-appropriate psychometric and neuropsychological tests. Sequential IQ studies have revealed a steady progressive decline in both verbal and full scale IQ in patients on the cranial radiation plus intrathecal chemotherapy treatment arm, while patients treated with HDMTX showed a slight increase in these measures. Thus, we have demonstrated significant adverse effects of cranial radiation and intrathecal MTX on intellectual function and found HDMTX alone to be less neurotoxic. We are also comparing the effects of these treatments on academic achievement. The standardized Wide Range Achievement Test (WRAT) has been used to assess academic achievement in patients 42 months following initiation of therapy. The WRAT assesses reading, spelling and arithmetic skills. Patients who received cranial radiation plus intrathecal MTX showed significant under achievement on reading ( $p < .05$ ), spelling ( $p < .05$ ), and arithmetic tests ( $p < .01$ ) when compared to individuals treated with HDMTX. Thus, in addition to a progressive decrement in IQ, patients treated with cranial radiation plus intrathecal MTX also manifest significant impairment of academic achievement.

## Publications

Balis FM, Mirro J, Reaman GH, Evans WE, McCully C, Doherty KM, Murphy RF, Jeffries S, Poplack D. Pharmacokinetics of subcutaneous methotrexate. *J Clin Oncol* 1988; 6:(in press).

Brouwers P, Moss H, Reaman G, McGuire T, Trupin E, Libow J, Tarnowski K, Bleyer W, Feusner J, Ruymann F, Miser J, Hammond D, Poplack D. Central nervous system preventive therapy with systemic high dose methotrexate versus cranial radiation and intrathecal methotrexate: Longitudinal comparison of effects of treatment on academic achievement of children with acute lymphoblastic leukemia. (abstr) *Proc Am Soc. Clin Onc* 1988;6:176.

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Arndt CAS, Balis FM, McCully CL, Jeffries SL, Doherty, K, Murphy R, Poplack DG. Bioavailability of low dose versus high dose 6-mercaptopurine. *Clin Pharmacol Ther* 1988;43:588-92.

Rothenberg ML, Mickley LA, Fojo AT, Cole D, Balis FM, Hamilton TX, Ozols RF, Poplack DG. Modulation of Dihydrofolate (DHFR) and multidrug resistance (mdr-1/P-170) genes as mechanisms of clinical drug resistance in patients with acute lymphoblastic leukemia (ALL). *Blood*(in press)



Moscow JA, Fairchild CR, Madden MJ, Ransom DT, Wieand HS, O'Brien EE, Poplack DG, Cossman J, Myers CE, Cowan KH. Expression of anionic glutathione-S-transferase and P-glycoprotein genes in human tissues and tumors. *Cancer Res* 1989;49:1422-8.

Colamonici OC, Quinones R, Rosolen A, Trepel JB, Sausville E, Phares JC, Gress R, Poplack D, Weber J, Schechter GP, Neckers LM. The beta subunit of the IL-2 receptor mediates interleukin-2 induction of anti-CD3 redirected cytotoxic capability in large granular lymphocytes. *Blood*, 1988, concise report, 71 (3);825-8.

Hockett RD, deVillartay J-P, Pollock K, Poplack DG, Cohen DI, Korsmeyer SJ. Human T-cell antigen receptor (TCR)  $\delta$ -chain locus and elements responsible for its deletion are within the TCR  $\alpha$ -chain locus. *Proc Natl Acad Sci USA*, 1988, 85;9694-8.

Colamonici, OR, Rosolen A, Cole D, Kirsch I, Felix C, Poplack DG, Neckers LM. Stimulation of the  $\beta$ -subunit of the IL-2 receptor induces MHC-unrestricted cytotoxicity in T acute lymphoblastic leukemia cells and normal thymocytes. *J Immunol* 1988;141(4);1202-5.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <b>Z01 CM 06880-12 PB</b>																
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           The clinical pharmacology of antineoplastic agents used in the treatment of pediatric malignancies is studied with emphasis on the role of pharmacologic monitoring and on both pre-clinical and clinical pharmacologic studies of Phase I agents. The clinical pharmacology of orally administered antileukemic agents has been evaluated and the limited bioavailability and variable drug levels of 6-MP achieved following oral administration has been documented. Studies are underway to determine the extent to which this phenomenon is the cause of treatment failure. Preclinical and clinical pharmacokinetic studies of a variety of new agents including Fazarabine, Piritrexim, and Thiotepla plus GM-CSF are in progress. A major effort of this project is to study experimental approaches to the treatment of CNS malignancy. A unique primate model is utilized to study the CNS pharmacokinetics of various intrathecally and intravenously administered chemotherapeutic agents; to evaluate the neurotoxicities of various CNS treatments; and to evaluate and screen newer CNS treatment modalities and drug schedules. Information gained from the studies with this model is then applied to the design of clinical treatment protocols. Protocols evaluating strategies such as prolonged intravenous 6-MP infusions and intravenous Thiotepla for brain tumors are under way. Clinical studies of intrathecal AZQ and continuous intrathecal 6-MP, approaches developed in this model, are in progress. Pre-clinical studies evaluating intra-CSF drug administration via indwelling drug delivery devices is under way. As part of the Pediatric Branch AIDS research effort, the Leukemia Biology Section is studying the clinical pharmacology of antiretroviral agents in children. The study of these agents is a natural extension of our work on the clinical pharmacology of anticancer drugs, since most of the antiretroviral agents are nucleoside analogs, similar to the antimetabolites used in the treatment of ALL. We have focused on several areas of research. An <i>in vitro</i> model utilizing a human CD4 positive cell line has been established to study the biochemical pharmacology of the dideoxynucleosides which are prodrugs that require intracellular activation to their corresponding triphosphorylated nucleotide. The CNS pharmacology of the antiretroviral is being systematically evaluated in our nonhuman primate model, to determine which agents may be most effective against the CNS HIV infection. We have also participated in the design of clinical trials of antiretroviral agents in children and performed detailed pharmacokinetic studies in the children treated on these trials.         </p>																		

Objectives:

1. To perform pre-clinical and clinical pharmacologic studies on new agents with particular emphasis on those being used to treat pediatric malignancies and those with potential activity against CNS malignancies.
2. To explore a subhuman primate model which provides repetitive access to the cerebrospinal fluid and allows detailed study of the pharmacology and neurotoxicity of chemotherapeutic agents used to treat CNS malignancy.
3. To study the CNS pharmacokinetics of currently employed and potentially useful CNS antineoplastic agents.
4. To perform pre-clinical pharmacologic evaluation of new anti-retroviral agents undergoing Phase I testing in children.

Methods Employed and Major Findings:A. Clinical Pharmacology of Antineoplastic Agents1. Clinical Studies on Thiotepa

We have evaluated the clinical pharmacology of Thiotepa in children with malignancy. Thiotepa is an active alkylating agent with a steeper dose response curve than cyclophosphamide. Our studies have demonstrated that substantial amounts of both thiotepa and its metabolite Tapa are present in the CNS following intravenous administration. This data indicates that this route of administration may be a more optimal one to approach CNS disease with this agent. As a result of these studies, a Phase I study of intravenous thiotepa in pediatric patients was undertaken and completed. The results suggested that systemically administered thiotepa may be a valuable agent for the treatment of CNS malignancies. In addition, this study demonstrated that thiotepa can be safely administered to pediatric patients at significantly higher doses (the MTD was  $65\text{mg/m}^2$ ) than those used conventionally in adults. In addition, *in vitro* studies of the activity of thiotepa and tapa against human CNS tumors have been performed using medulloblastoma and glioma cell lines. Both thiotepa and tapa show significant *in vitro* activity against these CNS tumor cell lines at drug concentrations achievable in patients at the dose recommended in our phase I trial.

Based on these findings the following studies are being pursued:

Phase II Trial of Intravenous Thiotepa in Pediatric Brain Tumors

A collaborative study of intravenous thiotepa at a dose of  $65\text{ mg/m}^2$  for pediatric patients with brain tumors has recently been opened for patient accrual as a multi-institutional study.

### Phase I trial of the Combination of Thiotepea and GM-CSF

High dose thiotepea, in combination with other agents, is being used as preparative therapy for autologous bone marrow transplantation for the treatment of brain tumors. Although promising clinical responses have been observed, the inability to repeatedly perform autologous bone marrow transplantation limits this therapeutic approach. Granulocyte-macrophage colony stimulating factor (GM-CSF) is one of several cloned hematopoietic growth factors which have been demonstrated to significantly modify the degree and duration of chemotherapy induced neutropenia. We have initiated a phase I protocol designed to evaluate the feasibility of administering escalating intravenous doses of thiotepea in conjunction with GM-CSF. In this study, patients will receive intravenous thiotepea on an every three week schedule starting at the MTD defined in our previous phase I study. GM-CSF is administered subcutaneously during the post-chemotherapy period. The thiotepea dose will be escalated (in 30% increments) with the aim of determining the highest dose of thiotepea which can be safely administered with adjunctive GM-CSF therapy. If effective, subsequent studies evaluating this approach in patients with CNS and other pediatric malignancies will be initiated.

#### 2. Phase I Studies

In addition to studies of Thiotepea we have performed Phase I studies on other agents including Fazarabine (Ara-AC), a new nucleoside analog with much broader antitumor activity in preclinical tumor screens than cytarabine (Ara-C), Pirarixim, a new nonclassical antifolate that is available in an oral formulation, and interleukin-2, which is discussed in the Childhood Leukemia Project Report.

#### 3. New Intrathecal Agents

Based on our work in the non-human primate model two new intrathecally administered agents have been investigated in clinical trials, intrathecal AZQ and intrathecal 6-MP. A third agent intrathecal 4-hydroperoxycyclophosphamide and the related compound, mafosfamide is being evaluated pre-clinically.

##### Intrathecal AZO (Diaziquone).

AZQ is a lipophilic alkylating agent designed for enhanced penetration of the blood-brain barrier. In preclinical studies, we demonstrated that following intravenous infusion, significant levels of AZQ were achieved in CSF. However, in subsequent clinical phase II studies evaluating parenteral AZQ for treatment of brain tumors, the systemic administration of this compound was found to be associated with severe, cumulative and dose-limiting hematologic toxicity. Because of the considerable preclinical data indicating that AZQ is active against a variety of CNS tumors as well as leukemias, we evaluated the possibility of administering AZQ intrathecally. Initially we studied the CSF pharmacokinetics of AZQ following intraventricular injection in our sub-human primates and found that ventricular and lumbar CSF drug exposure (AUC) were 20- and four- fold higher, respectively, than the CSF AUC achieved with intravenous administration of 80 times the intraventricular dose. The feasibility and safety of intraventricular AZQ was also

confirmed in the model. As the result of these studies, we developed a phase I/II trial of intrathecal AZQ which is currently in progress. Two dose schedules of AZQ are being evaluated in patients with refractory meningeal neoplasia, including standard bolus intrathecal administration of 1 mg twice weekly or a CxT schedule (0.5 mg intraventricularly every 6 hours x 3 doses). The CxT approach is designed to take advantage of the greater antitumor activity that we noted with this agent *in vitro* following prolonged drug exposure. To date, a total of 31 patients with refractory meningeal malignancy have been entered onto this protocol. Complete responses have been achieved in 13 patients, ranging from one to nine months in duration. No significant neurologic or systemic toxicity has been observed. These promising results in a group of heavily pretreated patients suggests a future role for intrathecal AZQ in the treatment of CNS leukemia and other meningeal malignancies.

#### Intrathecal 6-Mercaptopurine.

We have examined the feasibility of administering 6-MP by the intrathecal route. In initial studies in the nonhuman primate model we demonstrated that 6-MP could be safely administered by the intraventricular route. CSF 6-MP concentrations were found to decline biexponentially with  $t_{1/2}$ 's of 40 minutes and 2.8 hours. In addition, our results indicated that concentrations of 6-MP found to be cytotoxic *in vitro* against a variety of human tumor cell lines could be readily achieved in CSF at doses that are well tolerated. As an extension of these studies we recently initiated a clinical phase I trial of intrathecal 6-MP in patients with refractory meningeal malignancy. Both bolus administration (at a dose of 10 mg) and a CxT schedule (1 mg administered every 12 hours for 6 doses) are being studied. Complete remissions have been achieved in four of the nine patients treated on the bolus schedule. The remission durations range from two to five months. Entry onto the CxT arm of the study has only recently begun. Although preliminary, these data indicate that intrathecal administration of 6-MP is tolerable and suggest that this approach may eventually prove useful, not only for the treatment of overt meningeal leukemia, but also as CNS preventive therapy in childhood ALL.

#### Intrathecal 4-Hydroperoxycyclophosphamide/Mafosfamide.

The highly active alkylating agent, cyclophosphamide, is a prodrug, which must be converted by hepatic microsomal enzymes into 4-hydroxycyclophosphamide before expressing its antitumor effects. Because of this requirement for hepatic activation, cyclophosphamide is inactive *in vitro* and would not be an appropriate agent for regional administration. In contrast, 4-hydroperoxycyclophosphamide and mafosfamide, preactivated derivatives of cyclophosphamide, exhibit activity *in vitro* equal to that of 4-hydroxycyclophosphamide. 4-hydroperoxycyclophosphamide has demonstrated activity against a variety of malignant cell lines including L1210 leukemia, Burkitt's lymphoma, and breast cancer, and it is used for purging leukemic cells from human bone marrow prior to autologous bone marrow transplantation. We are currently investigating the possibility of administering 4-hydroperoxycyclophosphamide or mafosfamide intrathecally. In our nonhuman primate model intrathecal use of this compound was not associated with either acute or chronic neurotoxicity or with systemic toxicity. Identical results have been obtained with mafosfamide. The demonstration that cytotoxic levels of these agents can be achieved in CSF following intraventricular administration of a non-toxic dose

suggests that further study in the clinical setting is warranted. A clinical phase I trial of mafosfamide in patients with refractory meningeal malignancy is currently being prepared.

#### 4. Continuous Intrathecal Infusion

Intrathecal agents are currently administered by bolus injection, despite the fact that the most commonly used agents, MTX and cytarabine, are antimetabolites which have been shown to be more cytotoxic with prolonged exposure. In addition, because other intrathecal agents (AZQ, thiotepea) are cleared rapidly from the CSF following bolus injection, they must be given in higher doses to maintain a minimal cytotoxic concentration for any significant length of time. In some instances a CxT approach has been used to circumvent these problems. The ultimate extension of the CxT approach is to administer the drug by continuous infusion, an approach we are currently studying in our Rhesus monkey model. In previous studies in our laboratory, pharmacokinetic modeling with cytarabine demonstrated the potential pharmacokinetic advantages of continuous intrathecal administration in maintaining a minimal cytotoxic concentration in the CSF for a prolonged period with a much lower total dose. In addition, the chemical arachnoiditis frequently associated with intrathecal therapy has been linked with the high peak CSF concentrations following bolus injection. This can be avoided when the drug is given by low-dose continuous infusion. The Rhesus monkey model was adapted to enable us to perform these studies. A new technique was developed in which a cannula is inserted into the lateral ventricle and then attached to a subcutaneously implanted catheter with a reservoir which is attached to a portable infusion pump containing the drug to be studied. In preliminary studies we have found that with continuous infusion of MTX, ventricular CSF MTX concentrations are maintained at 1  $\mu\text{mol/L}$  for two- to three-fold longer than with the bolus dose, despite the fact that only one tenth of the total bolus dose was administered by infusion. Thus, these studies directly demonstrate the clear pharmacokinetic advantage for continuous intrathecal infusion. We plan to extend these studies to other agents including cytarabine, 6-MP, and AZQ and to move this approach rapidly to the clinic.

#### 5. Intrathecal Immunotoxin-monoclonal antibody studies

Immunotoxins, antibodies linked to peptide toxins, represent new potent and specific drugs potentially useful for treatment of malignancies, such as the administration of a cytotoxic anti T-cell ALL monoclonal antibody - Richin-A Chain conjugate for T-cell malignancies. The pharmacology and toxicity following intraventricular administration was assessed. The CSF clearance of the conjugate appears to be by CSF bulk flow. Laboratory studies indicate the CSF concentrations of immunoconjugate exceed the LD50 for T-ALL cells by 3 1/2 logs. Following intraventricular administration, a concentration exceeding the LD50 is maintained in the CSF for greater than 24 hours. No clinical neurotoxicity was observed in these studies. The significance of a mild eosinophilic pleocytosis is being evaluated. Based on these findings, clinical studies of this approach are being planned.

B. Clinical Pharmacology of Maintenance Therapy in ALL

Traditional maintenance therapy for ALL has consisted primarily of orally administered 6-MP and MTX. Although these drugs have been in use for over three decades, the clinical pharmacology of orally administered maintenance therapy has only recently been studied in detail. We have been studying the clinical pharmacology of drugs used in maintenance therapy. These studies are detailed in the *Leukemia Project Report*.

C. Clinical Pharmacology of Antiretroviral Agents

As part of the Pediatric Branch AIDS research effort, the Leukemia Biology Section is studying the clinical pharmacology of antiretroviral agents in children. The purpose of this project is to investigate the clinical pharmacology of both new and clinically available anti-AIDS drugs in children. Specifically we are studying 1) the pharmacokinetics of antiretroviral agents in order to determine the optimal route and schedule of administration and to establish correlations between pharmacokinetic parameters and both treatment response and toxicity; 2) the central nervous system pharmacology of existing and proposed AIDS therapies in order to predict the potential clinical efficacy against AIDS dementia complex; and 3) the biochemical pharmacology of the active nucleoside antiretroviral agents *in vitro* in a CD4 positive cell line in order to determine how to optimize intracellular formation of the active triphosphate nucleotides. An additional aim that relates to the clinical pharmacology of our new agent studies is to 4) study the clinical pharmacology of the nonclassical antifolates, TTX and piritrexim, which are now proposed for the treatment of *Pneumocystis carinii* pneumonia in patients with AIDS.

1. Pharmacokinetics of Antiretroviral Agents in Children

We have characterized the pharmacokinetics of AZT in 37 children with symptomatic HIV infection treated in the Pediatric Branch. These children were being treated on one of two phase I protocols utilizing either an intermittent (every 6 hour) or continuous infusion schedule of AZT. With intravenous bolus dosing the elimination of AZT in children was rapid and biexponential with half-lives of 14 and 90 minutes and a total clearance of 680 ml/min/m<sup>2</sup>. The major pathway of elimination appears to be the metabolic transformation of AZT to its 5'-glucuronide conjugate (GAZT). The renal clearance of 170 ml/min/m<sup>2</sup> suggested that the drug is both filtered and secreted by the renal tubule. There was considerable interpatient variation in the rate of drug elimination and there was no evidence of dose-dependency in the rate of AZT elimination. Oral bioavailability of AZT was also determined to be 68%. A simulation of the dose and schedule of AZT (180 mg/m<sup>2</sup> every 6 hour) proposed for children revealed that, with intermittent IV bolus dosing of AZT, plasma concentrations of AZT remain above the target level of 1 µmol/L for less than half of the dosing interval, and that the steady state trough concentrations are less than 0.2 µmol/L suggesting that this dose and schedule may be inadequate given the presumed importance of sustained continuous exposure to virostatic concentrations of AZT. For this reason we pursued an alternative approach utilizing the continuous infusion of AZT via a portable infusion pump. We studied 21 children treated on this schedule at one of four dose levels. Plasma concentrations of AZT were maintained above 1 µmol/L even at the lowest dose level, demonstrating a clear pharmacokinetic advantage for this schedule over

intermittent administration. This point was illustrated by a pharmacokinetic simulation which demonstrated that, using this intermittent schedule, a dose of 1,000 mg/m<sup>2</sup> every six hours would be required to maintain a minimum plasma concentration of 1 µmol/L. Pharmacokinetic parameters obtained on the continuous infusion schedule were similar to those obtained on the bolus AZT study. Drug clearance was age-related, especially when normalized to body weight with younger patients demonstrating more rapid clearance. The difference was less striking when clearance is normalized to body surface area, suggesting that dose should be calculated based on surface area rather than weight in future studies. In this Phase I study neutropenia was the dose-limiting toxicity; the degree of neutropenia appeared to be related to the plasma concentration of AZT. Patients who dropped below an ANC of 500/mm<sup>3</sup> during the first six weeks of therapy had significantly higher plasma AZT concentrations (mean 3.6 µmol/L) than those who remained above 500/mm<sup>3</sup> (mean 2.6 µmol/L). We have suggested that 3.0 µmol/L should be considered a toxic level on the continuous infusion schedule, pending more extensive studies. The identification of this toxic level along with the significant interpatient variability noted indicates a potentially important role for therapeutic drug monitoring in AZT therapy. Since the results of the clinical trial of continuous infusion AZT suggest efficacy for this schedule (see AIDS Project Report), two new approaches are currently being investigated to provide continuous exposure to AZT-- the continuous infusion of AZT subcutaneously and the development of an oral sustained-release formulation.

## 2. Central Nervous System Pharmacology of Antiretroviral Agents

Using our Rhesus monkey model we have systematically studied the CSF penetration of the antiretroviral agents and define those physicochemical properties that influence the degree of CNS penetration. Initially, the pyrimidine dideoxynucleosides AZT and ddC were studied in collaboration with the Clinical Pharmacology Branch. CSF penetration, as measured by the ratio of CSF to plasma drug concentration, was 21% for AZT and only 3% for dideoxycytidine. In contrast, when injected intraventricularly, no difference was noted in the CSF drug concentrations of these two agents, indicating that the difference in penetration was not due to a difference in the rate of clearance from the CSF. To determine the portion of the molecule that was responsible for this marked difference in penetration, we subsequently evaluated the penetration of dideoxythymidine, which had a CSF to plasma ratio of 30%, and azidodideoxycytidine which had a CSF to plasma ratio of 1%. These studies clearly indicate that the base (cytosine vs. thymine), rather than the 3'-substitution (azido group vs. none) on the sugar, determines the extent of CNS penetration. Of interest, the plasma protein binding and octanol/buffer partition coefficients of each of these compounds was also determined. None of the compounds was significantly protein bound. The azido group on the sugar resulted in a significant increase in the lipid solubility, but there was no apparent relationship between CSF/plasma ratios and lipid solubility. It appears, therefore, that a carrier-mediated process is primarily responsible for CNS entry of this class of drugs. As part of the phase I trial of continuous infusion AZT in children we measured simultaneous CSF and plasma steady state AZT concentrations in 21 children and found a CSF to plasma ratio of 24% - confirming the predictive ability of the Rhesus monkey model in studying antiretroviral agents.



This degree of penetration correlates with improvements in neurologic status of the patients treated on this trial.

### 3. Biochemical Pharmacology of Dideoxynucleosides

The effect of extracellular drug concentration and duration of exposure on the formation of the active intracellular triphosphate of ddC (ddCTP) is being investigated in the MOLT-4 human T-cell line *in vitro*. The intracellular metabolism of dideoxynucleosides is quantitated following exposure of the cells to radiolabeled parent drug. Thus far we have observed that levels of intracellular ddCTP increase proportionally to the extracellular concentration of the drug up to concentrations of 1  $\mu$ mol/L. Above this concentration, ddC is toxic to the cells. Formation of ddCTP is also enhanced by more prolonged exposure to the parent drug up to 24 hours, at which time, ddCTP levels plateau. Since the dose of ddC used clinically ranges from only 15 to 50 mg and peak plasma concentrations are well below 1  $\mu$ mol/L, the current dose and schedule do not result in saturation of the intracellular kinases responsible for the activation of ddC. Other factors that may affect or even enhance the the formation of ddCTP are being investigated using this system.

### 4. Treatment of P. Carinii Pneumonia with Nonclassical Antifolates

The nonclassical antifolate, TTX, originally designed and tested as an anticancer drug, has been shown to be efficacious in the treatment of *Pneumocystis carinii* pneumonia (PCP). We previously performed a phase I trial and pharmacokinetic study of TTX in children with refractory cancer, including development of a specific assay for the drug and identification of metabolic pathways. The bioavailability of oral TTX was studied in patients with AIDS and found to be 44%. Oral absorption did not appear to be saturable, as has been previously described for naturally occurring folates and MTX, suggesting that TTX is absorbed by a different mechanism. Plasma TTX concentrations nearly equivalent to those achieved with an intravenous dose were attained by administering an oral dose that was two-fold higher. We are currently measuring 24 hour drug concentrations in a large number of AIDS patients in an attempt to correlate this level with toxic reactions and response.

The phase I trial and pharmacokinetic study of another nonclassical antifolate, piritrexim, which is in progress, may also have applications for the treatment of PCP. This agent is already available in an oral formulation.

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<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 CM 06890-10 PB																								
PERIOD COVERED October 1, 1988, to September 30, 1989																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Lymphoma Biology and Epstein-Barr Virus																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 40%;">Ian Magrath</td> <td style="width: 30%;">Head, Lymphoma Biology Section</td> <td style="width: 10%;">PB, NCI</td> </tr> <tr> <td>Others:</td> <td>Ligita Novikovs</td> <td>Biologist</td> <td>PB, NCI</td> </tr> <tr> <td></td> <td>Melissa Adde</td> <td>Nurse Specialist (Research)</td> <td>PB, NCI</td> </tr> <tr> <td></td> <td>Bruce Shiramizu</td> <td>Medical Staff Fellow</td> <td>PB, NCI</td> </tr> <tr> <td></td> <td>Mary McManaway</td> <td>Biotechnology Fellow</td> <td>PB, NCI</td> </tr> <tr> <td></td> <td>Maya Moutot</td> <td>Visiting Associate</td> <td>PB, NCI</td> </tr> </table>			PI:	Ian Magrath	Head, Lymphoma Biology Section	PB, NCI	Others:	Ligita Novikovs	Biologist	PB, NCI		Melissa Adde	Nurse Specialist (Research)	PB, NCI		Bruce Shiramizu	Medical Staff Fellow	PB, NCI		Mary McManaway	Biotechnology Fellow	PB, NCI		Maya Moutot	Visiting Associate	PB, NCI
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BRANCH Pediatric Branch																										
SECTION Lymphoma Biology Section																										
INSTITUTE AND LOCATION National Cancer Institute, Bethesda, Maryland																										
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The major goal of this project is to elucidate the molecular consequences of the non-random chromosomal translocations (particularly 8;14 translocations) associated with the small non-cleaved (undifferentiated) lymphomas (SNCL) with a view to understanding the immediate causes of neoplastic behavior in these tumors and the determinants of geographic and clinical heterogeneity. Encompassed within this goal are our studies directed towards elucidating the nature of the association of Epstein-Barr virus (EBV) with the SNCL, and stemming from it, our investigations into the possibility that the molecular abnormalities can be used as a target for a tumor-specific treatment approach. Clinical studies parallel and complement these biological investigations as well as examining important chemotherapeutic issues including the use of dose intensity analyses to dissect the importance of various components and the duration of combination chemotherapy regimens, and to study prospectively the possibility and value of increasing dose intensity through use of colony stimulating factors.</p>																										

## PROFESSIONAL PERSONAL (CONTINUED)

Walter Goldschmidts	Biotechnology Fellow	PB,NCI
Abdulla Al-Nasser	Visiting Associate	PB,NCI
Vinay Jain	Visiting Associate	PB,NCI
Ayesha Jafri	Special Volunteer	PB,NCI
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Ronald Liebermann	Senior Investigator	FDA/PB,NCI

## OBJECTIVES AND FIELDS OF RESEARCH

Our goals include 1) to understand as much as possible of the molecular pathogenesis of the SNCL, and, ultimately, to use such ination to develop novel therapeutic approaches, and 2) to improve the management and treatment results of patients with childhood non-Hodgkin's lymphomas. Our ongoing areas of research fall into several overlapping categories:

- 1) *Molecular analysis of chromosomal breakpoint locations.*
- 2) *Use of the polymerase chain reaction (PCR) to detect translocations - a diagnostic and clinical tool.*
- 3) *Identification of a second genetic lesion in SNCL*
- 4) *Determination of the pathogenetic role of EBV.*
- 5) *Antisense regulation of c-myc translation in small non-cleaved lymphomas*
- 6) *Development of a small non-cleaved lymphoma xenograft model in nude mice.*
- 7) *Therapeutic Studies.*
- 8) *Collaboration with Cancer Centers in Less Developed Countries*

## SIGNIFICANCE

Biological Studies

The SNCL, although relatively rare tumors, have provided a crucially important model which has influenced all branches of oncology. It is probably safe to say that the cause and nature of neoplastic growth is better understood in the SNCL than in any other tumor. Our establishment of a library of cell lines derived from SNCL has been crucial to our ability to study the biology of these tumors and has benefited not only ourselves, but many other investigators.

Our studies are comprehensive, in that they encompass epidemiology, molecular pathogenesis, clinical correlates of molecular findings and therapeutic trials. The molecular categorization, in terms of the chromosomal breakpoint locations of these tumors, provides not only a new and considerably more precise epidemiological and diagnostic tool, but is also generating important leads to the understanding of the genesis of the chromosomal translocations, the mechanisms whereby the *c-myc* gene is deregulated, and the possible role of EBV in pathogenesis. Further, this new knowledge could lead to completely novel, tumor-specific treatment approaches as exemplified by our work with anti-intron antisense oligomers. While it is obviously too early to estimate their impact, such approaches, if successful, could confer a totally new perspective on cancer therapy.

Because of the paucity of pathogenetic ination in the vast majority of tumors it is probably only in the SNCL that such approaches can be seriously contemplated at present.

Our use of the highly sensitive PCR technique to identify breakpoint locations provides a new dimension on diagnostic techniques and the detection of minimal disease states. We have been able to detect the presence of a specific translocation in as few as 1 cell per million.

The demonstration that EBV has a direct role in the pathogenesis of the SNCL, and identification of the pertinent mechanisms would be of major importance to human viral oncology, and could also lead to new therapeutic approaches to EBV associated SNCL, which, once again, could provide a paradigm relevant to other tumors. Although discovered in 1964, the role of EBV in the pathogenesis of Burkitt's lymphoma has remained unclear ever since. Yet progress in understanding the biology and molecular biology of the virus, coupled to advances made in the molecular characterization of the SNCL, has provided an opportunity which did not previously exist, to elucidate the nature of the association of EBV with Burkitt's lymphoma.

### Therapeutic Studies

The demonstration that short duration, intensive chemotherapy provides effective treatment for patients with malignant lymphomas would provide major benefits in terms of less toxic cost, less hospital inpatient time and less disturbance to the lifestyles of patient and family. More importantly, however, we hope to demonstrate that patients with a high tumor burden will have an improved outcome. Our dose intensity analysis strongly suggests that in the SNCL, the dose rate is crucially important to outcome, and that only two to three cycles of chemotherapy are necessary. The demonstration that GM-CSF not only ameliorates the toxic side effects of chemotherapeutic agents, but permits an increase in dose intensity could provide a double benefit if the increased dose intensity could be shown to translate into a survival advantage. This approach could well be applicable to other rapidly growing neoplasms including most of the tumors of childhood as well as a number of adult malignancies. This approach could also be transferrable to the less developed countries which account for three quarters of the worlds population and 85% of the worlds children less than 15 years of age. In such countries, where supportive care is frequently inadequate, such that a high percentage of patients would die when the intensity of therapy is increased, decreased toxicity alone would almost certainly result in a marked improvement in survival. Our collaborative studies with centers in developing countries will permit the exploration of this concept.

### PROGRESS REPORT AND FUTURE DIRECTIONS

#### 1. Molecular Analysis of Chromosomal Breakpoint Locations

*Differences in the location of breakpoints on chromosomes 8 and 14 between endemic and sporadic tumors.*

Non-random chromosomal translocations, the most important being an 8;14 translocation, provide a critical element in the pathogenesis of the small non-cleaved lymphomas. Our studies of the significance of the breakpoint locations on chromosomes 8 and 14 have provided important ination. Burkitt's lymphoma has a much higher incidence in endemic regions (primarily equatorial Africa) than in other parts of the world (sporadic), but, as we have shown, there are a number of clinical and biological differences between these two s of the disease. The endemic variety is nearly always associated with EBV, and has a high frequency of jaw tumors while the sporadic variety is much less often associated with EBV (15%-20%) and uncommonly presents or relapses in the jaw. We have now demonstrated that these two forms of the disease are associated with molecular genetic differences, and have located the breakpoints to small regions in and

around the *c-myc* allele involved in the translocation. These studies on over 60 tumors have been performed primarily by Southern blot analysis, supplemented by S1 protection studies and Northern analysis in the case of cell lines. We have demonstrated that chromosomal breaks occur in the first intron of the *c-myc* gene in some 40% of sporadic tumors, but not at all, to date, in endemic tumors. The vast majority of sporadic tumors have breakpoints within or close to *c-myc*, whereas the majority of endemic tumors have breakpoints much further away from *c-myc* (referred to as "far 5' breakpoints subsequently).

We have also demonstrated that whereas in endemic tumors the majority of breakpoints fall outside the switch  $\mu$  immunoglobulin region on chromosome 14, approximately one third of sporadic tumors have switch  $\mu$  breakpoints, approximately twice as frequent. In addition, the majority of switch  $\mu$  breakpoints are associated with breakpoints within or close to the *c-myc* gene, although the reverse does not apply - non-switch  $\mu$  breakpoints are equally distributed between far 5' breakpoints and breakpoints in or close to *c-myc*. These findings have a number of important implications. Firstly, they complement our clinical observations, and together, they indicate that there are important biological and probably etiological differences between sporadic and endemic SNCL. Secondly, they strongly suggest that the mechanism of deregulation of *c-myc* differs in endemic and sporadic tumors. Breakpoints within or close to *c-myc* result in loss of the normal promoters, or of regulatory sequences which drive the promoters. Breakpoints outside the switch  $\mu$  region lead to the recognized immunoglobulin enhancer being on the same chromosome as *c-myc*, while switch breakpoints may result in alternate promoters or regulatory elements acting on the *c-myc* gene.

#### Molecular Epidemiologic Studies of Small Non-cleaved Cell Lymphomas

In addition to the more basic studies described above, we are planning to extend our molecular epidemiological investigations by characterizing small non-cleaved lymphomas from other world regions. We have so far established collaborations in Egypt, India, Turkey and South America for this purpose, and have trained or are training individuals from Egypt, Chile and Argentina in the required molecular techniques. We would particularly like to examine the patterns of breakpoint locations in tumors occurring in tropical and temperate South America to determine whether patterns con to those seen in tropical Africa versus temperate world regions, and to characterize the EBV association of these tumors. North African tumors are also of particular interest because of their high rate of EBV association (some 85%), while the apparently high incidence of jaw tumors in Turkey and some parts of India is also worthy of investigation at a molecular level in view of our findings with regard to endemic versus sporadic tumors.

#### Clinical Correlates of Breakpoint Locations

##### *Development of a Second Clonally Discrete SNCL in a Patient with HIV Infection*

As an example of a more pragmatic use to which Southern blot analysis of chromosomal breakpoint location can be put, we recently compared two SNCL developing in succession in a homosexual patient with HIV infection. We were able to show that the second tumor, which arose 3 years after the first, resulted from a second translocation which occurred in a different cell. This demonstrates that individuals at high risk for a particular tumor may develop a second tumor which, without molecular analysis, would be indistinguishable from a recurrence of the first. It also demonstrates that such tumors may respond to the same therapy used to treat the initial tumor. This phenomenon may well explain the small percentage of very late relapses (2 to 4 years after presentation) that occur in African Burkitt's lymphomas, and is relevant to lymphomas associated with HIV infection, although in this circumstance may be obscured by death from opportunistic infections.

### *Anatomical Location and Prognosis*

We are planning a collaborative study with the POG group to examine the significance of different subtypes of SNCL determined by the breakpoint locations on chromosomes 8 and 14. We are interested in determining if different mechanisms of deregulation of *c-myc* lead to different clinical presentations, or different responses to chemotherapy. If such correlates can be made, this information could prove to be of value to the design of treatment protocols.

### 2. Use of PCR to Detect Translocations - a Diagnostic and Clinical Tool

We have been able to detect breakpoint locations with PCR by using the repeat sequences within the switch  $\mu$  region as one of the amplicons (oligonucleotides). We have been able to distinguish different breakpoint regions within the *c-myc* gene by this method, and also, to detect one cell per million with such translocation. Recently we have been able to perform the same analysis on frozen fixed tissue. This technique should therefore permit the performance of molecular epidemiological studies on tissue already available in pathology departments, provides a highly specific diagnosis (based on the presence of a chromosomal translocation) and can be explored for its utility in the clinic as a means of detecting minimal quantities of disease before (e.g. in CSF) and after (residual disease) therapy.

### 3. Identification of a Second Genetic Lesion in SNCL

Recently, we have collaborated with Dr Mark Smulson of Georgetown University and assisted him in his studies of the enzyme poly ADP ribose polymerase (PARP). He has studied Southern blots made by us with probes prepared from a c-DNA clone of this gene. These studies have provided evidence that there is a second molecular lesion present on chromosome 13 in SNCL as well as other tumors such as lung and breast cancer. This lesion appears to be a deletion that extends into a pseudogene for PARP present on this chromosome. Evidence for the deletion has been found on one or both 13 chromosomes in all endemic tumors studied and in EBV negative, but not EBV positive sporadic tumors examined. These data suggest the presence of a suppressor gene on chromosome 13 which may be absent or non-functional in a subset of SNCL, and raise the possibility that there may be a genetic predisposition to SNCL.

### 4. Pathogenetic Role of EBV in Small Non-Cleaved Lymphomas

#### *Direct Examination of Influence of EBV on c-myc Expression*

In addition to making correlations between breakpoint location and EBV association, we are more directly studying the potential mechanisms whereby EBV could provide an essential pathogenetic element. One possible scenario is that latently expressed EBV genes influence *c-myc* expression directly, i.e. via the regulatory elements of *c-myc*. This is a likely possibility, since several latently expressed EBV genes are transactivators - i.e. influence the expression of other EBV genes. Indeed, the mechanism whereby EBV effects B cell transformation is likely to involve the transactivation of cellular genes - possibly even *c-myc*. We have hypothesized that structural changes in the *c-myc* gene may sometimes be sufficient in themselves to effect deregulation and neoplasia, but in other cases an effect of EBV on one or more of the *c-myc* regulatory elements may be essential. We plan to examine the possible effect of EBV on *c-myc* by several different experimental approaches.

### *Use of a c-myc Construct Attached to a Reporter Gene and Gel-retardation assays*

We have made a number of plasmid constructs containing either the entire presumptive *c-myc* regulatory region (of normal origin), or various components of this region, and attached them to a reporter gene (luciferase). This construct will be transfected into EBV positive and negative cell lines, and also cell lines containing plasmid constructs expressing specific EBV genes, in order to determine a) whether the expression of luciferase (now governed by *c-myc* sequences) is altered by EBV and specific EBV genes, and b) whether it is possible to detect differences in the pattern of proteins bound to specific regions of the *c-myc* regulatory region (e.g. by exonuclease assays, gel retardation assays, or direct detection of specific proteins such as EBNA 1 bound to specific DNA fragments). The presence of proteins on specific *c-myc* regions in EBV positive Burkitt's lymphoma cells but not EBV negative cells would indicate that EBV gene products transactivate *c-myc*.

### *Use of Antisense Oligomers Directed Against Specific EBV Latent Genes*

Finally, in view of our experience with antisense oligomers directed against regions of the *c-myc* gene (see below), we have commenced experiments in which the effect of antisense molecules directed against specific latent EBV genes is being studied in Burkitt's lymphoma cell lines containing EBV. This approach is realistic because only a small number of latent genes are expressed in Burkitt's lymphoma (and only latent infection, i.e. non-permissive for virus particles) is compatible with malignancy. If an EBV gene is essential to the malignant state, antisense treatment will result in failure of the cells to proliferate. In the case of EBNA 1, necessary for EBV plasmid maintenance, an alternative possibility is that EBV genomes may be lost from the cell line. Such findings, in addition to their theoretical importance, could have therapeutic implications for EBV associated tumors.

### 5. Antisense Regulation of *c-myc* Translation in Small Non-cleaved Lymphomas

We have pursued our objective of attempting to demonstrate that knowledge of the molecular abnormalities of a tumor may lead to novel treatment approaches by exploring the possibility of developing a means of specifically inhibiting the translocated *c-myc* gene in SNCL, while not affecting the *c-myc* gene of normal cells. We have chosen to use an antisense oligomer directed against 1st intron *c-myc* sequences, since these are not present in normal *c-myc* transcripts, but are present in SNCL which have breakpoints 3' of the normal promoters of *c-myc*, when transcription is initiated at the cryptic promoter site within the first intron. Using a 21 base oligomer derived from an intron region immediately adjacent to the second exon, we have been able to inhibit proliferation completely in cell lines (ST486 and JD38), in which intron sequences are present in the mRNA, but not in lines (KK124 and MC116) lacking such intron sequences. Other oligomers, such as the equivalent sense sequence, have no effect on either cell line. We have also shown a reduction in *c-myc* protein in this cell line when treated with the anti-intron antisense oligomer.

We have also collaborated with Dr Brian Huber of Burroughs Wellcome, who has constructed a plasmid containing the same 21mer antisense sequence linked to the human metallothionein gene promoter in association with a selectable marker (the *neo* gene). We have developed stably transfected cell lines by growth in high concentrations of the antibiotic G418, and are attempting to derive clones in which the antisense molecule can be induced by cadmium. If this is successful, many experiments requiring large numbers of cells should become feasible. Among such experiments will be a detailed analysis of the effects of antisense inhibition of *c-myc* on the expression of cellular



proteins (initial screening will probably be carried out by 2-dimensional gel electrophoresis), and the use of the stably transfected lines to tumors in nude mice (see below). We should then be able to determine whether efficient inhibition of the tumor *c-myc* in vivo (by feeding the mice with a heavy metal inducer) can result in tumor regression.

Other approaches to the in vitro and in vivo inhibition of tumor cell growth that will be explored include the packaging of antisense in liposomes to prevent enzyme degradation, the use of derivatized antisense molecules, e.g. phosphorothioate oligomers, or oligomers linked to an intercalating agent, and the use of retroviral vectors containing antisense molecules attached to a suitable promoter. Such molecules may be essential to our continued exploration the possible therapeutic use of antisense molecules in our nude mouse xenograft system described below.

#### 6. Development of a Small Non-cleaved Lymphoma Xenograft Model in Nude Mice

We have developed a xenograft model of SNCL in nude mice, which we plan to make use of in many different ways.

##### *Effect of Growth Factors on the Proliferation of SNCL cells, and Exploration of the Role of c-myc in the induction of Growth Factors or their Receptors*

We have demonstrated that different clones derived from the same parent cell line have reproducibly differing tumorigenicity in nude mice. The clones differ both with regard to the frequency of successful "takes" although the rate of growth of individual tumors does not differ. We are studying a variety of factors of potential importance to oncogenicity, including the level of expression of various relevant genes, such as *c-myc*, CD23, transferrin, interleukin 6 and *bcl-2*; the level of surface expression of leukocyte adhesion molecules; and the ability of cell lines to synthesize and respond to growth factors. In this regard, using serum-deprived cell lines, we have shown (with Dr Giovanna Tosato) that SNCL are able to respond to both autocrine and paracrine growth factors. This is consistent with observations that SNCL lymphomas occur frequently in sites where rapid proliferation of normal cells is occurring e.g. the developing tooth buds in African children, and the pubertal or lactating breast in women.

Preliminary experiments suggest that the oncogenicity of different cloned cell lines correlates with expression of *c-myc*. Since growth of the cell lines as tumors appears to be independent of host factors, if there is a true correlation between *c-myc* expression and oncogenicity, the possibility that *c-myc* induces either growth factor receptors or growth factors would be an important possibility to consider. The pathogenetic significance of constitutive expression of *c-myc* in such a circumstance would be clear. The effect of *c-myc* on the production of growth factors or the response to growth factors will therefore be investigated by measuring growth factor response or production in cells in which expression of *c-myc* has been inhibited with antisense or anti-immunoglobulin (see below), and cells in which proliferation but not *c-myc* expression has been inhibited by theophylline or drugs which inhibit DNA synthesis.

##### *Use of the Model to Examine the Effect of Antisense Molecules on Tumor Growth*

This nude mouse model should also be invaluable for the exploration of novel treatment approaches, including the use of *c-myc* anti-intron antisense molecules packaged in various different ways (see above).

## 7. Therapeutic Studies

Our clinical and therapeutic studies have provided a wealth of information on childhood lymphomas. One of the original objectives of our work was to identify factors indicative of prognosis, and this information is now being used in defining patient eligibility for the latest protocol. Analysis of the results of patients treated according to protocol 77-C-145 with a cyclophosphamide/doxorubicin/vincristine/prednisone combination followed by a prolonged (42h) infusion of methotrexate at the nadir of myelosuppression have shown that tumor burden (as measured by clinical stage, serum LDH or IL-2 receptor level) and dose intensity are the most important prognostic factors.

### *Recent Findings*

Recently, we have completed analyses of the associated disease patterns and relevance to treatment of testicular and bony sites of tumor. In addition, we have demonstrated that obtaining multiple bone marrow samples is as important to the determination of bone marrow involvement in young patients with lymphoma as it is in adults - a finding which goes against accepted dogma. Having previously demonstrated that tumor burden appears to be the most important prognostic factor, we have shown that circulating interleukin-2 receptor levels provide an objective measure of tumor burden more specific than serum lactate dehydrogenase (LDH) and more accurate than stage. We believe that such objective markers of tumor burden provide a more valid means of assessing comparability of different published patient series, and have shown that the results of protocol 77-C-145 are similar to those of Total Therapy B when LDH is used to divide the patients into different subgroups. We plan to develop additional assays for the presence of elevated levels of serum protein molecules expressed by lymphoma cells (e.g. common acute lymphocytic leukemia antigen and various B and T cell associated antigens) in order to determine whether such assays provide even more specific and precise measures of tumor burden.

### *Dose Intensity Analysis*

We have largely completed an analysis of dose intensity in protocol 77-C-145 (largely a question of cycle duration in this case). We have shown that this kind of analysis provides information which is highly pertinent to the design of treatment protocols. For example, the dose intensity of drugs in cycles 1 and 2 is highly significantly associated with treatment outcome, although such an association is not present when subsequent cycles are considered. Similar conclusions are arrived at when only the SNCL are examined, and since these tumors represent the majority of the patients, it is reasonable to conclude that additional therapy beyond three cycles does not contribute to outcome in the SNCL. Such a conclusion cannot at present be generalized to the lymphoblastic lymphomas, and indeed, may well not apply in this case.

In this analysis we have been unable to demonstrate a significant association of treatment outcome with adriamycin, vincristine and prednisone dose rate in the SNCL, indicating that cyclophosphamide and methotrexate are the most important drugs in this tumor. Preliminary analysis suggests that this is not so for the lymphoblastic lymphomas, where vincristine and adriamycin may be very important drugs.

Of additional interest was the finding that both partial responders and patients with bone marrow involvement had lower average dose intensities than other patients. There was significant overlap between these groups of patients. This raises the possibility that such patients would benefit from more rapid initiation of subsequent therapy cycles. Delays incurred in

their treatment resulted from delays in white blood cell count recovery. Armed with this ination, it may prove possible to develop new treatment strategies designed to overcome this problem.

#### *Development of a Treatment Regimen for Patients with non-Lymphoblastic Lymphomas*

We have developed a new protocol for the treatment of patients with non-lymphoblastic lymphomas. The high risk component of this protocol is based on two regimens piloted in clinical trials. The first of the pilot protocols, was a modified, slightly intensified version of 77-C-145. The second pilot protocol, instituted as a forerunner of the new protocol consisted of a the new drug combination consisting of Etoposide, Ifosfamide and high dose Cytarabine. This regimen was shown to be active, even in patients previously treated with the 77-C-145 regimen and probably, therefore, resistant to the combination to be used as the alternating arm of the new protocol. Low risk patients will be treated with a simpler regimen.

#### Low risk patients.

Patients with localized or completely resected abdominal disease, will be treated with only 3 cycles of therapy based on modified 77-C-145 - i.e cyclophosphamide/doxorubicin/vincristine alternating with an infusion of methotrexate over 24 h. The intent with low risk patients is to reduce treatment duration as much as possible (half the number of cycles given previously) while ensuring that more than 90% of patients continue to achieve long term survival. The new protocol will involve no radiation and should avoid the potential side effects which are sometimes encountered when high cumulative doses of some drugs (e.g doxorubicin) are received.

#### High risk patients

All patients not eligible for the low risk protocol will be treated with a regimen consisting of alternating cycles studied in the two pilot protocols. In view of empirical data emanating from Germany, where patients are treated effectively with only 12 weeks of therapy, and our own data suggesting that there is no advantage to prolonged durations of treatment, patients will receive only 4 cycles of therapy.

#### Evaluation of the effectiveness of GM-CSF in ameliorating toxicity and permitting increased dose intensity.

In view of the anticipated high degree of toxicity of this protocol, and the consequent likelihood that delays in therapy will be incurred with consequent reduction in dose intensity, we have designed a randomized study in one arm of which patients will receive GM-CSF. This should provide several opportunities. These include the possibility to determine: 1) whether GM-CSF will lessen the degree of myelosuppression and consequently the incidence of fever and infection in patients treated with the new protocol. 2) Whether lessened myelosuppression will translate into an increased dose intensity (i.e mgs of drug/M<sup>2</sup> administered per week). 3) Whether the increased dose intensity will translate into a survival advantage. In addition, patients in the control arm will also receive additional therapy.

The high grade B cell lymphomas represent a particularly appropriate model in which to attempt to shorten the interval between therapy cycles with GM-CSF because of the rapid regrowth of these tumors, which is one reason for chemotherapy failure. The chosen protocol design enables us to make both a retrospective comparison of survival in equivalent patient groups treated according to protocol 77-C-145 and also a comparison of survival in patients treated with and without GM-CSF. Although rather more patients will be

required for the latter, the precise number will depend upon the degree of difference, if any, between the two arms.

#### 8. Collaboration with Cancer Centers in Less Developed Countries

We have developed collaborations in less developed countries in order to further assist local scientists and clinicians in the characterization and treatment of lymphoid neoplasms occurring in these geographic regions. We are interested in exploring the influence of different environmental circumstances on the frequency of various subtypes of leukemias and lymphomas, and have a particular interest in characterizing the SNCL occurring in these regions at a molecular level (see below). We have provided assistance in the development of therapeutic protocols in India and have provided advice and in some cases reagents for the phenotypic characterization of the lymphoid neoplasms in both India and Egypt. Data regarding socioeconomic status, occupation and rural/urban residence is being routinely collected.

Recently we have shown that in Egypt, lymphoblastic lymphoma is very uncommon in children (less than 10% of all lymphomas). On the other hand, based on the phenotyping of 186 cases, 50% of the cases of acute lymphoblastic leukemia in this country are of T cell type, regardless of age. Common acute lymphoblastic leukemia is reciprocally reduced in frequency (39%), while null cases and B cases accounted for the remaining cases. We are pursuing this finding by carrying out further phenotypic analysis in Egypt and molecular analysis in this laboratory. We are interested in determining which T cell receptor genes are rearranged, and whether we can find evidence of breakpoints within the T cell receptor genes.

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<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 CN 06891-01 PB
PERIOD COVERED October 1, 1988, to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Solid Tumors</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Marc E. Horowitz	Senior Investigator PB, NCI
Others:	Linda L. Weaver	Nurse Specialist (Res) PB, NCI
COOPERATING UNITS (if any) Radiation Oncology Branch, NCI (E. Glatstein); Surgery Branch, NCI (S. Rosenberg); Clin Pathology, NCI (R. Elin); Cardiology, NHLBI (R. Bonow)		
LAB/BRANCH Pediatric Branch		
SECTION		
INSTITUTE AND LOCATION National Cancer Institute, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>Research into new therapeutic strategies for the treatment of pediatric solid tumors is focused on bone and soft-tissue sarcomas including Ewing's sarcoma, peripheral neuroepithelioma, rhabdomyosarcoma and osteosarcoma. These common pediatric tumors remain diagnostic and therapeutic challenges for which new approaches are needed. The sarcomas also serve as an excellent "model system" for the exploration of strategies and hypotheses that have broad applicability to both pediatric and adult solid tumor oncology. The overall goal of these protocols is to learn how to use drugs that have been determined to be active in the pediatric sarcomas with sufficient intensity to maximize their therapeutic potential.</p> <p>Previous Pediatric Branch protocols have demonstrated a very high response rate for intensive vincristine, adriamycin and cyclophosphamide in newly diagnosed sarcoma patients (83-C-73) and a high level of activity for ifosfamide, mesna and etoposide in those with recurrent tumors (85-C-154). The current front-line sarcoma protocol (86-C-169) is studying the integration of the ifosfamide, mesna, etoposide combination with intensive vincristine, adriamycin, cyclophosphamide, and local irradiation. In an effort to circumvent the major toxicity associated with this protocol, myelosuppression, we are studying the hematopoietic growth factor rh-GM-CSF in a randomized trial to determine whether its use will decrease the myelosuppression, related delays and toxicity (88-C-165). We are also studying the iron chelator ICRF-187 in a randomized trial (89-C-07) in patients on the sarcoma protocol to learn whether it will protect the heart from adriamycin induced myocardial damage. These studies of ICRF-187 and rh-GM-CSF are unique in that they are the only ongoing front-line trials of these promising new approaches in pediatric solid tumor patients.</p>		

## CLINICAL STUDIES

Protocol 83-C-73 - Treatment of Patients With Ewing's Sarcoma With Central Axis Primaries and/or Metastatic Disease, Rhabdomyosarcoma, and Other High Risk Soft Tissue Sarcomas

In 1983 Pediatric Branch study 83-C-73 was initiated to test the response to intensive VAdRC and local irradiation with consolidation by total body irradiation (TBI) and autologous bone marrow reconstitution. Seventy-five patients were entered and treated at the NCI over a three year period. The diagnoses were: Ewing's (n=32), PN (n=14), rhabdomyosarcoma (n=24), and undifferentiated sarcoma (n=5). Thirty-six patients had metastatic disease at diagnosis and the majority central axis primary lesions. Over 90% of the patients responded completely to irradiation and chemotherapy. Despite the excellent initial responses the survival and event-free survival for the entire group at approximately four years is 49% and 29% respectively. A major difference was seen for those with or without metastatic disease at presentation. Event free survival at approximately three years is 25% versus 49% respectively. Event free survivals for those with Ewing's, PN and rhabdomyosarcoma are not significantly different. The method used to obtain local control was, in 80%, a surgical biopsy and local irradiation. The actuarial local control rate at approximately three years was 70%. In 10 patients local and distant failure was noted simultaneously. Three failed with local disease only. Of 13 patients with local failure, three had metastatic disease at diagnosis and nine had tumors of the trunk for which complete resection was not an option.

Protocol 86-C-169 - A Pilot Study for the Treatment of Patients With Metastatic and High Risk Sarcomas and Primitive Neuroectodermal Tumors

This protocol is designed to define the initial response rate, overall effectiveness and toxicities of a combination of intensive vincristine, adriamycin and cyclophosphamide with the new combination ifosfamide and etoposide for patients with sarcomas. Eligible patients are those less than 25 years of age with Ewing's sarcoma, peripheral neuroepithelioma and primitive sarcoma of bone, metastatic unresectable rhabdomyosarcoma or spindle cell sarcoma. Treatment commences after a surgical biopsy. A complete surgical resection is not attempted unless this can be easily accomplished without mutilating surgery and a major delay in the initiation of chemotherapy. Induction chemotherapy is delivered over twelve weeks prior to the initiation of radiotherapy. This "neo-adjuvant" design is supported by the results of study 83-C-73. Radiotherapy is delivered after week 12 chemotherapy. The primary site is treated to a field encompassing the original tumor volume with approximately 45 Gy. An additional 15 Gy is delivered to a coned down field.

To date there have been 39 protocol entries with the following diagnoses: Ewing's sarcoma (n=16), PN (n=10), primitive sarcoma of bone (n=5), rhabdomyosarcoma (n=4), other soft tissue sarcomas (n=4). Twenty-eight patients had central axis lesions and 22 metastatic disease at diagnosis. The numbers are too small and the duration of follow-up too short to judge the efficacy of this treatment. Response to the four pre-irradiation induction chemotherapy cycles (VAdRC-IE-VAdRC-IE) have, with the exception of two patients, been excellent (> 50% tumor reduction). There have been 18 protocol failures with progressive tumor in 15 and 3 deaths from toxicity (sepsis 1, cardiomyopathy 1, bleeding 1). The toxicity of this protocol has been significant. 96% of treatments have been associated with grade IV neutropenia (AGC nadir < 500) and in 59%, infection. Although the majority of infections have been fever, without a source, the incidence of sepsis is 7% with one toxic death from septic shock. The myelosuppression has resulted in delays in treatment. Instead of the scheduled treatments every 21 days the average interval between treatments is 25 days. During or after radiation therapy the



average interval between treatments is 28 days. Cardiac toxicity has also been significant. The patients are prospectively evaluated by radionuclide angiography (MUGA). There have been two episodes of clinically apparent cardiomyopathy; one resulting in death. The majority have a drop in MUGA scan ejection fraction to the lower levels of normal as they approach the cumulative 550 mg/m<sup>2</sup> called for in the protocol. In some patients adriamycin was discontinued early because of the ejection fraction changes.

Although it is premature to judge the efficacy of this treatment as a general statement it is unlikely that significant gains will be realized by the introduction of new drugs if they result in a degree of toxicity that precludes their optimal utilization. We are therefore developing ways to decrease myelosuppression and cardiac toxicity in order to allow maximal benefit from VAdRC-IE by increasing dose intensity over time.

Protocol 88-C-165 - A Randomized Placebo-Controlled Trial of Recombinant Human Granulocyte-Macrophage Colony Stimulating Factor in Pediatric Patients Following Intensive Combination Chemotherapy

This protocol was initiated as a randomized double blind study of rh-GM-CSF in patients on the sarcoma protocol to learn whether it will significantly reduce myelotoxicity and resultant delays in therapy. Patients received rh-GM-CSF at 10 uG/kg subcutaneously daily beginning 24 hours after completion of the chemotherapy regimen and continuing for 10 days. Seven patients have been entered in the study. The results were "unblinded" when it became clear that the effects of the agent precluded a true double blind comparison. From the 6 patients receiving the GM-CSF we have learned that it will not obviate neutropenia. In 20 cycles analyzed, the GM-CSF was discontinued after ten days with an absolute neutrophil count still below 500 in every case. From these initial patients the protocol has been amended in order that the study be randomized but not blinded. The GM-CSF dose has been increased to 15 uG/kg daily through day 19 from the initiation of the chemotherapy cycle. It will be continued until the absolute neutrophil count remains above 500 for 48 hours. Studies elsewhere have demonstrated that GM-CSF may decrease the duration of neutropenia if not the nadir. This new schedule will test this.

Protocol 89-C-07 - A Phase III Study of ICRF-187 (Bisbiodoxopiperazine, ADR-529), an Adriamycin Cardioprotector, in Pediatric Sarcoma Patients

Patients on the sarcoma protocol are randomized to receive ICRF-187 with adriamycin or adriamycin alone to learn whether this iron chelating agent will decrease the significant incidence of clinical and subclinical adriamycin associated cardiomyopathy. The patient's cardiac function is monitored closely with radionuclide angiography which is the endpoint for the study. The study opened this spring and four patients have been entered.

Protocol 87-C-68 - A Randomized Trial of Pre-Surgical Chemotherapy Vs. Immediate Surgery and Adjuvant Chemotherapy in the Treatment of Non-Metastatic Osteosarcoma - A Pediatric Oncology Group Phase III Study

The Pediatric and Surgery Branches of the NCI have a long history of studying osteosarcoma. Since 1981 studies have been carried out in collaboration with the Pediatric Oncology Group as the "Multi-Institution Osteosarcoma Study (MIOS)". The Pediatric Branch participation in this effort was essential for the completion of the study published in 1986 by Link et. al. in the New England Journal which demonstrated the value of adjuvant chemotherapy in osteosarcoma. Fully 50% of the randomized patients were treated at the NCI. The current study is testing the relative merits of immediate surgery versus neo-adjuvant chemotherapy. As the majority of osteosarcoma patients have resectable tumor at diagnosis important questions are adjuvant in nature and

must be addressed with phase III studies. The numbers of patients required for such studies necessitate multi-institution collaborations. Investigators from the NCI have been intimately involved with the design, conduct and analysis of the MIOS studies.

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Pizzo PA, Horowitz ME, Poplack DG, Hays DM, Kun LE. Solid tumors of childhood. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. Cancer principles & practice of oncology. 3rd ed. Philadelphia: JB Lippincott Company, 1989;1612-70.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 00650-34 RO

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

## Service Radiation Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)

PI: Eli Glatstein Branch Chief ROB, NCI

Others:	J. Bader	Chief, Clinical Radiotherapy Section	ROB, NCI
	T. DeLaney	Senior Investigator	ROB, NCI
	A. Raubitschek	Senior Investigator	ROB, NCI
	B. Kelly	On-Site Coordinator	ROB, NCI
	J. Grieg	Chief Technologist	ROB, NCI

## COOPERATING UNITS (if any)

## LAB/BRANCH

Radiation Oncology Branch

## SECTION

Radiation Therapy Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

10

## PROFESSIONAL

4

## OTHER

6

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this project is to provide expert radiotherapy, consultation, and treatment for patients of the Clinical Center, including patients admitted to services other than the ROB. Support is given to the Medicine Branch, Surgery Branch, Pediatric Branch, NCI/Navy Medical Oncology Branch, Neurosurgical Service, Endocrine Service, and others.

Project DescriptionProfessional Personnel Engaged on the Project:

J. Rowland	Nurse Specialist	CNS, CC
R. Smith	Clinical Nurse	CNS, CC
L. Walton	Clinical Nurse	CNS, CC
L. Dachowski	Clinical Nurse	CNS, CC

Methods Employed

Formal and informal consultation with referring physicians and application of radiotherapy where appropriate with x-rays and electrons in accordance with standard radiotherapy practice, as well as modified programs when necessitated by concomitant adjuvant therapies.

Major Findings

Just under 700 patients were seen in formal consultation this year. In addition, between 400 and 500 telephone conversations provided ad hoc advice on treatment for a variety of problems and general information, including nursing management and follow-up for radiation therapy related problems. Approximately three visits per month from nursing staff to observe delivery of radiation therapy and simulation process. Approximately 450 patients will be treated this fiscal year with most of these being protocol patients in the Radiation Oncology Branch, or on collaborative studies.

Proposed Course

To continue.

Publications

1. Finkelstein E, Glatstein E. Seduced by oxygen, Int J Rad Oncol Biol Phys 1988;14:205-7.
2. Lawrence TS, Urba WJ, Steinberg SM, Sundeen JT, Cossman J, Young RC, Glatstein E. Retrospective analysis of stage I and II indolent lymphomas at the National Cancer Institute, Int J Rad Oncol Biol Phys 1988;14:417-24.
3. Bader JL, Glatstein E. External beam radiation therapy of gliomas. In: Kornblith P, Walker M, eds. Advances in neuro-oncology. Mt. Kisco, New York: Futura Publishing Company, 1988;347-86.
4. McKenna WG, Bonomi P, Barnes MM, Glatstein E. Malignancies of the thymus. In: Roth JA, Ruckdeschel JC, Weisenburger TH, eds. Thoracic oncology. Philadelphia: WB Saunders, 1989;466-77.

5. Turrisi AT, Glatstein E. Principles of combined radiation therapy and chemotherapy. In: Moss WT, Cox JD, eds. Radiation oncology: rationale, technique, results. St. Louis: CV Mosby, 1989;68-82.
6. Findlay PA, Gorrell CR, D'Angelo T, Glatstein E. Lactation after breast radiation Letter to the Editor . Int J Rad Oncol Biol Phys 1988;15:511-2.
7. Glatstein E. Hemoglobin, tissue hypoxia, and radiation therapy [Question-Answer]. JAMA 1988;260:3508.
8. Glatstein E, Jett JR, Scoggin CH. Your role in lung cancer management. Patient Care 1989:56-72.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06310-10 R0

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Surgery Versus Radiation Therapy in Treatment of Primary Breast Cancer

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: K. L. Straus Senior Investigator, ROB, NCI

## COOPERATING UNITS (if any)

## LAB/BRANCH

Radiation Oncology Branch

## SECTION

Clinical Radiotherapy Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

6

## PROFESSIONAL:

3

## OTHER:

3

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to determine whether a breast-conserving treatment program of limited surgery and definitive radiation offers equivalent local control and survival to mastectomy in patients with early stage breast cancer. After work-up confirms localized disease, patients are randomly assigned to either primary surgery or primary irradiation. Patients treated with mastectomy are offered breast reconstruction. All patients undergo complete axillary node removal; those patients with pathologically positive lymph nodes, and those with negative lymph nodes who are estrogen receptor negative receive chemotherapy.

Project Description

## Professional Personnel Engaged on the Project:

H. McDonald	Senior Surgeon	SB, NCI
D. Danforth	Senior Investigator	SB, NCI
K. Cowan	Head, Med. Brst. Cancer Sect.	MB, NCI
W. Schain	Clinical Care Consultant	Rehab. Med., CC
N. L. Gerber	Chief, Rehab. Medicine	Rehab. Med., CC
T. d'Angelo	Cancer Nursing Specialist	CNS, CC
M. Merino	Surgical Pathologist	LP, DCBD, NCI
S. Steinberg	Head, Bio. & Data Manage. Sect.	BDMS, NCI

**Objectives:** If survival and recurrence data obtained with treatment that preserves a cosmetically acceptable breast are comparable to those obtained with radical surgical procedures, such treatment will probably be more acceptable to most women with localized breast cancer. Availability of an effective alternative to mastectomy may encourage women to seek medical attention with earlier, hence more curable, cancers. The cosmetic and functional results of local treatment will be carefully evaluated. The psychological, sexual and sociological impact of mastectomy vs. lumpectomy and radiation will be noted. Ability to combine aggressive chemotherapy with either local treatment in node positive patients and node negative, ER negative will also be assessed.

Methods Employed

Patients with stage T1-T2, N0-N1, M0 primary untreated breast cancer are candidates for the study. They will be randomized to receive either lumpectomy, axillary dissection and radiation therapy or total mastectomy with axillary node dissection. Patients receiving mastectomy will be offered breast reconstruction. Patients with pathologically positive lymph nodes, and ER negative patients with negative lymph nodes will receive chemotherapy.

Major Findings

This study has been active for 10 years. Currently, 256 patients have been entered, of whom 128 have randomized to mastectomy, and 128 to radiation. Median follow-up is 64 months. No differences have been seen as yet between the surgery arm and radiation arm in terms of overall survival (83% and 90% at 60 months respectively). There have been 15 local/regional recurrences in the radiation arm. (Eleven/fourteen in-breast-only failures were salvaged by mastectomy.) Nine local/regional recurrences have occurred on the mastectomy arm.

Significance to Biomedical Research and the Program of the Institute

The study is intended to determine whether breast conserving treatment

(lumpectomy and radiation therapy) is equivalent to radical surgery as treatment for early stage breast cancer. If this is the case, this treatment option should be much more acceptable to the majority of women. It is conceivable that the availability of such non-mutilizing treatment would encourage women to seek medical attention sooner, and therefore present with more curable disease.

#### Proposed Course

The study is ongoing, but will probably close within this calendar year.

#### Publications

Levy SM, Herberman RB, Lee JK, Lippman ME, d'Angelo T. Breast conservation versus mastectomy: distress sequelae as a function of choice, JCO 1989;7:367-75.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06320-10 RO

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Response of Mammalian Cells to Chemotherapy Drugs

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	J. B. Mitchell	Senior Investigator	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Cook	Associate	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

## COOPERATING UNITS (if any)

## LAB/BRANCH

Radiation Oncology Branch

## SECTION

Experimental Phototherapy Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

6

## PROFESSIONAL:

4

## OTHER:

2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several chemotherapy agents with proven utility such as anthracyclines, bleomycins, alkylators, neocarzinostatin, noble metal derivatives, VP-16, and hypoxic radiosensitizers are being studied. The detoxification mechanisms, modification of cellular response by altered intracellular redox status and oxygen metabolism in sensitive and resistant cells are of interest to the area of cancer treatment and directly related to our studies. Deleterious species produced by the antineoplastic drugs and cellular response to these species, as well as sulfhydryl containing compounds as they relate to metabolism, activation, and detoxification of antineoplastics. It has been demonstrated that depletion of glutathione levels either by directly conjugating or inhibition of de novo synthesis results in sensitization of cells by adriamycin, bleomycin, cisplatin, VP-16, alkylators, and hypoxic radiosensitizers. Alternatively, increasing glutathione levels by providing direct precursors results in protection of cells from the above reagents. Rescue of cells after treatment by supplying glutathione directly by modifying the molecule such that it becomes membrane permeable is being studied. Timing of delivery of the rescue agents, how the rescue agents interact with other biochemical pathways, cellular clearance and in vivo clearance are being investigated.

### Project Description

Objective: The objective of this project is to determine the importance of biochemical modulation of selected cellular redox compounds upon chemotherapeutic drug cytotoxicity.

### Methods Employed

In vitro cell culture and in vivo murine tumor models will be exposed to the various reagents mentioned above and assayed by conventional clonogenic assay, dye markers, tumor dose response, and survival advantage. In the in vivo studies, both thymic and athymic mouse are available to investigate murine and human tumor response. Standard biochemical enzyme assays, synthetic organic chemistry techniques, high performance liquid chromatography, and molecular biology techniques will and are being used.

### Major Findings

Cell killing is enhanced for adriamycin, cisplatin, bleomycin, VP-16, alkylating agents and hypoxic radiation sensitizers when glutathione levels are lessened in cells. Likewise, there is greater marrow effect when glutathione is modulated downward by several methods. Further, there is tissue differences as to response to depletion. Bone marrow may ultimately be more resistant to glutathione depletion than quickly growing tumors. Exogenous glutathione applied the mice treated with cisplatin has been protective. Ongoing studies will seek to if such an effect exists in tumor-bearing animals.

### Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding of drug-induced resistance and provide potential means of overcoming such resistant clones. Likewise, work is accumulating that may allow for differentiating normal tissue and tumor response to antineoplastic drugs by manipulating, in part, the redox status of cells.

### Proposed Course

To continue to explore the best means of modifying chemotherapy response by manipulation of redox cycles.

### Publication

None.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>			PROJECT NUMBER  ZO 1 CM 06321-10 RO
PERIOD COVERED <b>October 1, 1988 to September 30, 1989</b>			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Radiosensitization and Chemosensitization of Aerated and Hypoxic Mammalian Cells</b>			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
P.I.:	J.B. Mitchell	Senior Investigator	ROB, NCI
Others:	A. Russo	Senior Investigator	ROB, NCI
	J. A. Cook	Staff Fellow	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI
COOPERATING UNITS (if any)			
LAB/BRANCH <b>Radiation Oncology Branch</b>			
SECTION <b>Radiation Biology Section</b>			
INSTITUTE AND LOCATION <b>NCI, NIH, Bethesda, Maryland 20892</b>			
TOTAL MAN-YEARS: <b>4</b>		PROFESSIONAL: <b>2</b>	OTHER: <b>2</b>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>A major portion of this study has dealt with the importance of cellular redox systems such as glutathione (GSH) and related enzymes to the cell's defense against ionizing radiation and chemotherapy drugs. Recent studies have clearly shown that inherent GSH levels do not significantly contribute to the radiation response. This statement is substantiated by our work using drugs that result in GSH depletion to &lt;5% of control or elevation to 2 fold. Under these conditions, radiosensitivity was affected modestly (~10%). GSH levels were shown to markedly influence the extent of hypoxic cell radiosensitization by nitroimidazoles; however, 8799, an nitroimidazole sensitized independent of GSH levels.</p> <p>GSH modulation was found to significantly alter the chemotherapy response particularly for drugs such as melphalan and cisplatin. Particular attention was directed toward the development of an automated drug sensitivity (MTT assay) to screen a large panel (30 cell lines) of human lung cancer cell lines to determine if a relationship between chemosensitivity and GSH levels exists. Basically, the results showed that cell lines (small cell) low in GSH were more sensitive to cisplatin, melphalan, and adriamycin than cell lines 2-3 fold higher in GSH (non-small cell). The patterns of chemosensitivity observed correlated with what is seen clinically. These studies warrant further investigation as to the possibility of using GSH levels as a predictor of tumor response to chemotherapy drugs. GSH levels from fresh human tumor biopsies are being accumulated to test this hypothesis.</p>			

### Project Description

**Objective:** The objective of the proposed project is to obtain a better understanding of the nature of lesions and processes leading to cell reproductive death and to study the inter-relationships of factors which influence radiosensitivity and chemosensitivity, with an emphasis on their implications for the clinic.

### Methods Employed

In vitro cell reproductive integrity will be assayed by the single cell plating techniques for attached cells. Cells will be exposed to radiation or selected chemotherapy drugs, either under aerated or hypoxic conditions. Cellular GSH will be measured by spectrophotometric methods and cellular levels altered by drugs that specifically modulate the GSH cycle. Particular attention will be placed toward optimizing flow cytometric assays for GSH determination of fresh human tumor biopsy material.

### Major Findings

No relationship was found between chemoresistance and radiation sensitivity. GSH levels were found to significantly influence the response of cells to chemotherapy drugs. GSH is currently being studied and considered as a predictor of tumor cell response to chemotherapy drugs. Data are being accumulated regarding GSH levels of human tumors.

### Significance to Biomedical Research and the Program of the Institute

These results show that modulation of tumor cell GSH can influence the cellular response to chemotherapy drugs. Agents such as buthionine sulfoximine (BSO) which inhibits GSH synthesis are being considered for chemotherapy clinical trials. Depletion of GSH by BSO could improve tumor treatment for selected chemotherapy drugs provided normal tissue toxicity is not also increased. GSH levels in human tumors and normal tissues are being accumulated to determine if GSH levels in tumors are higher than normal tissues.

### Proposed Course

More studies will be conducted at the cellular level on a more efficient means of GSH modulation. A major effort will be directed toward the measurement of GSH (and related enzymes) in human tumor and normal tissue.

### Publications

1. Mitchell JB, Biaglow JE, Russo A. Role of glutathione and other endogenous thiols in radiation protection, *Pharmac Ther* 1988;39:269-74.
2. Mitchell JB, Russo A, Carmichael J, Glatstein E. Glutathione as a predictor of tumor response. In: Chapman JD, Peters LJ, Withers HR, eds. Prediction of tumor treatment response. New York: Pergamon Press Inc, 1989;157-74.

3. Carmichael J, Mitchell JB, DeGraff WG, Gamson J, Gazdar AF, Johnson BE, Glatstein E, Minna JD. Chemosensitivity testing of human lung cancer cell lines using the MTT assay, *Br J Cancer* 1988;57:540-7.
4. Carmichael J, Park JG, DeGraff WG, Gamson J, Gazdar AF, Mitchell JB. Radiation sensitivity and study of glutathione and related enzymes in human colorectal cancer cell lines, *Eur J Cancer* 1988;24:1219-24.
5. Mitchell JB. Glutathione modulation and cancer treatment, *ISI Atlas of Science* 1988;2:155-69.
6. Brown JM, Hall EJ, Hirst DG, Kinsella TJ, Kligerman MM, Mitchell JB, Travis EJ, Valeriote F. Chemical modification of radiation and chemotherapy, *Am J Clin Oncol* 1988;11:288-303.
7. Hall EJ, Astor M, Bedford J, Borek C, Curtis SB, Fry M, Geard C, Hei T, Mitchell JB, Oleinick N, Rubin J, Tu A, Ullrich R, Waldren C, Ward J. Basic radiobiology, *Am J Clin Oncol* 1988;11:220-52.
8. Carmichael J, Mitchell JB, Friedman N, Gazdar AF, Russo A. Glutathione and related enzyme activity in human lung cancer cell lines, *Br J Cancer* 1988;58:437-40.
9. Mitchell JB, Cook JA, DeGraff WG, Glatstein E, Russo A. Glutathione modulation in cancer treatment: will it work?, *Int J of Radiat Oncol Biol Phys* 1989;16:1289-95.
10. Cook JA, Russo A, Pass HI, Iype S, Mitchell JB. Use of monochlorobimane for glutathione measurements in hamster and human tumor cell lines, *Int J Radiat Oncol Biol Phys* 1989;16:1321-24.
11. Phillips TL, Mitchell JB, DeGraff WG, Russo A, Albright N, Rajpal R. Modification of SR 2508 sensitization in hypoxic V79 cells by manipulation of glutathione levels, *Int J of Radiat Oncol Biol Phys* 1989;16:1335-9.
12. DeGraff WG, Russo A, Gamson J, Mitchell JB. Evaluation of nitroimidazol hypoxic cell radiosensitizers in a human tumor cell line high in intracellular glutathione, *Int J Radiat Oncol Biol Phys* 1989;16:1021-4.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06329-09 R0

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Clinical Radiation Physics Service

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. van de Geijn Radiation Physicist ROB, NCI

Others: R. Miller Radiation Physicist ROB, NCI  
K. Yeakel-Orr Dosimetrist ROB, NCI  
F. Harrington Biomed. Engineering Tech. ROB, NCI  
To Be Appointed Radiation Physicist ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This section continues to provide expert physical and technological support for radiation treatment. This support consists of routine calibration and quality assurance of all radiation equipment and includes special dosimetry studies, computer-assisted treatment planning, and the design and development of special equipment tailored to special clinical needs. Regular checking of dosimetric and technical set-up aspects of radiation treatment will continue.

1. The improvement of the quality assurance (QA) program for the three Varian accelerators (Clinacs 4, 18, and 20) and the Scanditronix Microtron M22 is an ongoing effort. A new quality assurance detector using five ionization chambers has been integrated into the QA program. This device consolidates output, energy and symmetry checks and will be useful for electrons as well as photons.
2. Adaptation of the radiation equipment and special supporting equipment for patient treatment and its implementation is a continuing effort, continually adjusted also to the needs of the ongoing and new clinical research programs.

3. The Microtron has been repaired and acceptance testing has been completed.
4. The computer programs for clinical radiation treatment planning are being rewritten in C-language for implementation on a Macintosh II system. This innovation promises portability, and easy implementation by and for other institutions. Support of intracranial implants continues to be of particular interest in this regard.
5. Supporting patient treatment and evaluation of clinical research.

### Project Description

#### Personnel:

E. Lamoreaux	Computer Specialist	ROB, NCI
Huchen Xie	Computer Specialist	ROB, NCI
Junwen Chen	Radiation Physicist	ROB, NCI

**Objectives:** To ensure highly flexible and quality physics support for radiotherapy.

#### Methods Employed

The locally developed highly efficient system for daily and periodic quality assurance is continually used for monitoring the performance of three linear accelerators, the Microtron, the simulator, and the CI scanner. Special mechanical supports and measuring devices are used to quantify the position of patients and to improve the reproducibility of daily patient set-ups. The data acquisition for treatment planning have been simplified and improved. Considerable efforts have been invested in the dosimetry of intraoperative, total-body, and total-skin radiotherapy.

The Section continues to provide non-routine in vivo patient dosimetry by means of thermoluminescent dosimeters and diodes. Such ad hoc measurements are usually concerned with doses to sensitive organs, and are sometimes crucial to the continuation of a treatment technique.

#### Major Findings

This is a continuing project, developing in part in line with developing or new clinical research. Beam monitoring locally developed and other quality assurance support jigs enable daily monitoring of output, beam flatness, symmetry, and alignment of light field and x-ray fields for all three linear accelerators. The method allows simple documentation of performance. Our system continues to impress visitors. The dosimetry of photon beam total-body irradiation, as well as that of total-skin electron beam irradiation for mycosis fungoides, has stabilized.

The most important contribution in computer-assisted treatment planning is the availability of routine interactive optimization and routine multi-slice imaging of dose distributions superimposed on CT scans. An important aspect is the capability to image irregular fields shaped by individualized specially defined shielding blocks. This is of essential interest in the treatment of soft-tissue sarcomas and cancers of the esophagus.

The use of locally designed and developed equipment and methodology continues to be a major factor in quality control of equipment, methodology and treatment documentation. This is especially important in view of the generally highly complex clinical studies in this Branch. Reliability of treatment delivery is being improved by implementation of a computer controlled hand-held bar code reader system, developed in-house by Robert Miller.

#### Significance to Biomedical Research and the Program of the Institute

The improvements in quality assurance, patient positioning, and treatment planning are essential as a basis for optimal patient treatment and for meaningful evaluation of treatment protocol studies. The CT scanner is now the principal source of patient data for treatment planning.

#### Proposed Course

1. Continuation of adaptation of the computer programs to the new radiation machines, with emphasis on an updated system based on the Macintosh II.
2. Special attention to the quality assurance aspects of the Microtron, currently under re-installation.
3. Integration of alternative imaging systems such as MRI and PET into the updated treatment planning system.

#### Publications

None.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 CM 06330-09 RO

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Radiation Field Modeling and Computerized Treatment Planning

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2

PROFESSIONAL

1

OTHER

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

This is ongoing research and development. The capability to calculate the distribution of absorbed dose produced by photon beams and electron beams of the most general characteristics is fundamental to radiotherapy. The radiation field model has been described before. It takes as a basis the empirical distributions along three mutually perpendicular reference lines in a "master field." This concept is applied to the beam-modifying devices as well. One virtue of this approach is that it requires few experimental data and thus can be implemented very easily.

Existing computerized treatment programs are being revised and rewritten for use on a portable system. (This activity is in fact a continuation of # Z01 CM 06331-08 RO which is, for this reason, terminated as a separate project.)

### Project Description

Objectives: To extend unified calculative models for the description of absorbed dose produced by beams of ionizing radiation, including photon beams as well as electron beams, as a basis for computer-assisted treatment planning, with special attention to high energy x-ray and electrons.

### Methods Employed

Special attention will be paid to verification of the model for the revitalized Microtron, to the 6 MV and 21 MV x-ray beams as well as the electron beams. For this purpose, the newly updated Therados RFA-7 radiation field scanner is proving very useful.

### Major Findings

In x-ray dose field modeling, the description of electron transport correction has proved to be highly significant especially in high energy x-ray treatment with small fields in the thorax. Two publications are in preparation. The new electron beam model is both simpler to implement and has been shown to be more accurate than any other published model. All of these results are being incorporated in a new clinical treatment planning system built around a Macintosh II computer. The latter development has been held up by software problems.

### Significance to Biomedical Research and the Program of the Institute

The range of validity of the dose field model determines the potential range of applicability of the clinical treatment planning program. In turn, the latter determines the degree of refinement in radiation treatment that can be scientifically documented. Current development could also become attractive for dissemination into the radiotherapy community, and improve the exchangeability of treatment documentation in clinical trials. Cooperation with a commercial organization providing hundreds of community hospitals has been approved and will shortly be initiated.

### Proposed Course

- 1) This project is to be continued, with the emphasis of inhomogeneities in photon and electron beams. In regard to electron beams, the influence of inhomogeneities needs further experimental work and algorithmic implementation.
- 2) Implementation on a Macintosh II portable system is to be continued. (See Z01 CM 06378-03 R0.)

### Publications

None.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>			PROJECT NUMBER  Z01 CM 06351-07 RO
PERIOD COVERED October 1, 1988 to September 30, 1989			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Response of Mammalian Cells to Halogenated Pyrimidines			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)			
P.I.:	J.B. Mitchell J.A. Cook	Senior Investigator Staff Fellow	ROB, NCI ROB, NCI
Others:	A. Russo W. DeGraff J. Gamson	Senior Investigator Biologist Biologist	ROB, NCI ROB, NCI ROB, NCI
COOPERATING UNITS (if any)			
LAB/BRANCH Radiation Oncology Branch			
SECTION Radiation Biology Section			
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892			
TOTAL MAN-YEARS 3	PROFESSIONAL: 2	OTHER 1	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews			
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>When certain halogenated pyrimidines such as bromodeoxyuridine (BrdUrd) and iododeoxyuridine (IdUrd) are incorporated into cellular DNA, cells become more sensitive to ionizing radiation and chemotherapy drugs. This observation has led to several clinical studies over the years and recently at the NCI to evaluate whether selective sensitization of tumors could be achieved by IdUrd infusion followed by radiation. An important question arises in these studies regarding the extent to which the drug is incorporated into cells. Work continues to develop techniques to quantify IdUrd incorporation into tumor and normal tissues. The IdUrd monoclonal antibody has proven useful in flow cytometry studies to accurately predict the labeling index (proportion of cells in S phase) and the laboratory is currently involved (with the clinical IdUrd study) in assessing this potentially important clinical parameter. Future studies will involve image analysis of tumor sections to gain insight as to spacial labeling patterns within the tumor. Quantitation of IdUrd replacement in tumor cell DNA is ongoing. We have been able to show that as the percentage of IdUrd replacement of thymidine increases, so does the extent of radiosensitization. We have demonstrated that IdUrd incorporation sensitizes cells to fission spectrum neutrons thereby opening the possible utility of this approach for neutron radiotherapy.</p>			

### Project Description

**Objectives:** To quantitate the amount of IdUrd in tumor vs. normal tissue by flow cytometry, HPLC, and image analysis. With these techniques, optimal timing schedules of incorporation for maximum differential radiosensitization will be determined. Determine if IdUrd incorporation sensitizes cells to neutron irradiation.

### Methods Employed

A monoclonal antibody for IdUrd and HPLC assays will be used to quantitate incorporation of IdUrd in tissues. Standard cell survival techniques have been used. Image analysis will be performed using a fluorescent microscope linked to laser excitation and computer image analysis systems.

### Major Findings

Positive identification of cells in tumor and normal tissue that had incorporated BrdUrd and IdUrd has been made using the monoclonal staining technique. A linear relationship was found between IdUrd replacement and radiosensitization. IdUrd incorporation was found to enhance fission spectrum neutron irradiation.

### Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding as to quantities and timing of IdUrd required to radiosensitize cells from tumor and normal tissue in a clinical setting. This parameter may be useful in selecting appropriate treatment approaches.

### Proposed Course

Continue work on cellular quantitations of IdUrd. Evaluate cell survival of other mammalian cells to halogenated purines and pyrimidines and work out timing of incorporation for maximum differential sensitization in *in vivo* models. The influence of biological response modifiers on IdUrd incorporation will be studied.

### Publications

1. Mitchell JB. Potential applicability of non-clonogenic measurements to clinical oncology, *Radiation Res* 1988;114:401-14.
2. Phillips TJ, Bodell WJ, Uhl V, Ross GY, Rasamussen J, and Mitchell JB. Correlation of exposure time, concentration and incorporation of IdUrd in V-79 cells with radiation response, *Int J Radiat Oncol Biol Phys* 1989;16:1251-55.
3. Atcher RW, DeGraff WG, Moore M, Grdina DJ and Mitchell JB. Halogenated pyrimidines as radiosensitizers for high LET radiation, *Radiation Res* 1989;117:351-5.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 06352-07 R0
PERIOD COVERED <b>October 1, 1988 to September 30, 1989</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Relaxation Agents for NMR Diagnostic Imaging</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	O. A. Gansow	Senior Investigator ROB, NCI
Others:	M. W. Brechbiel	Chemist ROB, NCI
COOPERATING UNITS (if any)		
LAB/BRANCH <b>Radiation Oncology Branch</b>		
SECTION <b>Inorganic and Radioimmune Chemistry Section</b>		
INSTITUTE AND LOCATION <b>NCI, NIH, Bethesda, Maryland 20892</b>		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.1	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>Nuclear Magnetic Resonance (NMR) imaging is a most powerful method for the non-invasive diagnosis of disease. A fundamental limitation of the technique derives from the fact that images are constructed from T1 relaxation time measurements of protons in the various biological "compartments". If T1 values for differing soft tissue types are similar, the types will not, in general, be resolvable in the images. A potential method for improving this situation is the development of relaxation agents which specifically alter T1 relaxation rates in tissues where they may be concentrated.</p> <p>We have recently shown that metal chelates are useful as an NMR contrast agent in myelography and cisternography. We have prepared a new and superior contrast agent, Gadolinium (Gd)DOTA. This chelate has recently been shown to be more stable <u>in vivo</u> than Gd(DTPA).</p> <p>This year, we report the preparation of new chelating ligands for linkage of contrast agents to proteins. An effective, efficient synthesis of 1-p-nitro-benzyl DOTA has been completed.</p>		

Project Description

## Professional Personnel Engaged on the Project:

J. Frank

Clinical Associate

DR, CC

Objectives: We proposed to construct paramagnetic molecules that localize in certain biological compartments in order to reduce T1 relaxation times of water in the area. We plan to attach paramagnetic metal chelates to proteins found to localize where desired in the body. The idea is that since paramagnetics alter local T1 values, by concentrating them in differing tissue types, we could induce resolution in NMR images. For example, paramagnetic labels attached to blood proteins, which circulate freely blood, thus allowing imaging of cardiac function and blood flow. A second example would be to label tumor associated monoclonal antibodies. In recent work done in this Section, it has been proven possible to localize radioisotopes attached to antibodies in tumors by using metal chelates.

To accomplish this goal, we must produce chelating agents which complex Gadolinium or other paramagnetic metals, and which can be linked to proteins. Our recent synthesis of 1-p-nitrobenzyl DOTA realizes this objective.

Methods Employed

Bifunctional metal chelates capable of securely binding paramagnetic metals like iron, chromium or Gadolinium have been prepared and attached to the proteins described above. The effect of these paramagnetic relaxation agents on T1 values have been measured by conventional inversion, recovery methods. The chelate, Gd(DOTA), is being tested as an MRI contrast agent (CA) by examination of relaxation times in aqueous media and in tissues excised from appropriate animals. In vivo studies of the MRI of CA enhanced tumors in mice are also in progress.

Major Findings

Studies have now shown that Gd(DTPA) may be attached to antibodies or albumin without affecting the biological properties of the proteins. Results of T1 studies show that many paramagnets must be attached to one protein to have an effect in vivo.

Use of Gd(DTPA) as an NMR contrast agent has demonstrated that MRI myelography and cisternography may be practically useful in the clinic.

We have prepared kilogram quantities of a new contrast agent Gd(DOTA) for use in humans.

In addition, considerable effort has been expended in devising methods for linkage of Gd(DOTA) to proteins to serve as a T1 relaxation agent. By consideration of the di-N-hydroxysuccinimide ester of di-N-protected-ethylenediaminediacetic acid with p-nitrobenzylethylenediamine, the resultant cyclic diamide was obtained. When reduced with diborane and subsequently tetraalkylated with bromoacetic acid, the new bifunctional chelate, 1-p-nitrobenzyl DOTA was prepared. The ligands have been shown to form exceptionally stable complexes with contrast imaging agents like Gadolinium (III).

#### Proposed Course

The efficacy of 1-p-benzyl DOTA Gadolinium (III) in inducing T1 relaxation in tissues will be investigated both in vitro and in vivo.

#### Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 CM 06353-07 R0

PERIOD COVERED  
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Metal Chelate Conjugated Monoclonal Antibodies for Tumor Dx & Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	O. A. Gansow	Senior Investigator	ROB, NCI
Others:	S. M. Mirzadeh	Expert	ROB, NCI
	M. Brechbiel	Chemist	ROB, NCI
	T. McMurry	Senior Staff Fellow	ROB, NCI
	G. Pippin	Staff Fellow	ROB, NCI

COOPERATING UNITS (if any)  
Johns Hopkins Medical School, Baltimore, MD (M. Strand); Argonne  
National Laboratory, Argonne, IL (R. W. Atcher)

LAB/BRANCH  
Radiation Oncology Branch

SECTION  
Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION  
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	4.3	PROFESSIONAL:	1.3	OTHER:	1.0
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CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Tumor-associated monoclonal antibodies are potential therapeutic agents as selective carriers of cytotoxic agents to malignant cells. We are testing this hypothesis in several animal model systems: one is a tumor virus induced leukemia of mice; another is human tumor xenographs in nude athymic mice.

The cytotoxic agents being employed are various radionuclides. Their relative efficacy when conjugated to monoclonal antibodies is being assayed and compared to that of monoclonal antibodies alone or conjugated to toxins. The several radionuclides chosen for study span the range of nuclidic properties available, thus Copper-67 represents a weak, short range, low energy beta emitter, Yttrium-90 is a long range, high energy beta emitter, Bismuth-212 is a short-lived, alpha emitter and Lead-212 provides both short and long range beta emissions and the subsequent alpha emission of its Bismuth-212 daughter. The syntheses of the chelating agents required for linkage of these isotopes to antibody is now complete. Protocols for labelling antibodies are nearly so. The first results from therapy studies with Yttrium-90 are in hand.

The new chelating agents synthesized for the therapy project have proven useful for imaging of malignancies in humans by use of the Indium-111 radionuclide. Imaging with Lead-203, the potentially most useful gamma imaging radionuclide available, is under investigation.

These studies will provide for human medicine a basis for design of rational therapy of malignancies by selectively targeting cytotoxic agents to tumors, as well as metastases and as well will allow diagnostic imaging of malignancies.



Project Description

## Professional Personnel Engaged on the Project:

D. Colcher	Senior Investigator	LCMB, NCI
T. Waldmann	Chief	MEI, NCI

Objectives: The specific goal of these studies is to investigate in vitro and in animal tumor models the therapeutic efficacy of radionuclides attached to tumor-associated monoclonal antibodies. These studies encompass the synthesis of new bifunctional chelates designed for therapy employing a variety of radioisotopes and radiation types.

Methods Employed

Methods for covalently conjugating metal isotopes in bifunctional chelates to monoclonal antibodies are being devised and developed. The inorganic chemistry of new complexing agents for metal isotopes thought to be useful in tumor diagnosis or therapy is being explored. The objectives of the research must thereby of necessity include: (a) the synthesis and characterization of new bifunctional chelates and their metal complexes, both before and after protein conjugation; (b) the evaluation of currently available chelates for use as carriers of isotopes familiar in clinical environments (e.g., Tc-99M) and of less common, but potentially serviceable radionuclides (e.g., Ga-68, In-111, Pb-212, Bi-212, Y-90); (c) the development of chemical procedures (protocols) for routine and reproducible preparations of rigorously stable radiometal chelate conjugated monoclonal antibodies which retain their inherent biological specificity and activity; and (d) the use of animal models for investigating the stability in vivo of metal labeled antibodies.

Major Findings

We report this year major progress in developing the chemistry required for irreversibly linking the radionuclides Lead-212, Bismuth-212, Copper-67, and Yttrium-90 to monoclonal antibodies. The stability of DTPA chelates for Yttrium-90 was reported in several publications. Based on this, two protocols for treatment of leukemia and lymphoma in patients have been approved to begin this year.

1. The fundamental chemistry of DOTA chelates of lead and bismuth were investigated and reported recently as the first step in a careful, arduous and lengthy selection of a suitable ligand structure for complexation of the Lead-Bismuth-212 alpha emitters. DOTA chelates seemed useful, so the new chelating agent 1-p-isothiocyanatobenzyl-DOTA has been prepared and tested with the alpha emitter Bismuth-212. In vivo tissue distribution studies show that this is the first known chelate of Bismuth to be stable in vivo and thus useful for radioimmunotherapy. Investigations of the Lead-212 chelates are in progress.
2. The chelating agent 1-p-SCN-MX-DTPA was prepared and tested in vivo with Yttrium-90. It is stable and useful for clinical tests of

radioimmunotherapy with Yttrium-90, as reported in the publications.

3. The use of Indium-111 D1PA linked to antibody B72.3 for imaging of human tumors in vivo was accomplished this year. Clinical doses were prepared and clinical protocols implemented in the radiopharmacy at the NIH.
4. A study of chelating agents for Copper-67 is in the final stages. A ligand has been selected for preparation with a sidearm for linkage to antibody. Synthesis of this ligand will be complete this summer.

#### Significance to Biomedical Research and the Program of the Institute

The ability to attach metals to antibodies is significant for several reasons. It enables one to diagnose and detect cancer using radioactive metals in nuclear medicine tests, or using paramagnetic metals to enhance nuclear magnetic resonance images. The ability to attach particle emitters to antibodies opens up site specific therapy using a variety of radioactive metals which can be selected to maximize cell killing while sparing normal tissue. Finally, it appears that the bifunctional chelates currently being investigated have little effect on the viability and specificity of the antibodies, thus preserving their function.

#### Proposed Course

Studies of the therapeutic efficacy of the several radionuclides now under investigation are in progress employing: 1) a model for leukemia in which normal mice have been infected with Rauscher leukemia virus; and 2) a human xenograph solid tumor model in mice. To this will soon be added a rabbit model for malignancies in the CNS and a mouse model for ovarian carcinoma. Based on these studies, we will be able to select the most appropriate radionuclide for radioimmunotherapy of the specified disease to be treated.

Radiobiology studies of relative in vitro therapeutic efficacy and dosimetry will be performed.

Protocols for diagnostic imaging with Copper-67 and Lead-203 will be implemented both to provide diagnostic information and to allow dosimetric calculations for therapeutic doses of radiolabeled antibodies.

Since protocols for production of clinical doses of chelate linked Yttrium-90 labeled antibody are in place, treatment of lymphoma, leukemia and colon cancer with radiolabeled antibody will be underway this year.

#### Publications

1. Rosselli M, Schlom J, Gansow OA, Raubitschek A, Mirzadeh S, Brechbiel MW, and Colcher D. Comparative biodistributions of yttrium- and indium-labeled monoclonal antibody B72.3 in athymic mice bearing human colon carcinoma xenographs, *Journal of Nuclear Medicine* 1989;30:672-82.

2. Kozak RW, Raubitschek A, Mirzadeh S, Brechbiel MW, Junghans R, Gansow OA, and Waldmann TA, Nature of the bifunctional chelating agent used radioimmunotherapy with yttrium-90 monoclonal antibodies: critical factors in determining in vivo survival and organ toxicity, Cancer Research 1989; 49:2639-44.
3. Blend MJ, Greager JA, Atcher RW, Brown JM, Brechbiel MW, Gansow OA, and Das Gupta TK, Improved sarcoma imaging and reduced hepatic activity with indium-111-SCN-Bz-DTPA linked to MoAb 19-24, Journal of Nuclear Medicine 1988;1810-16.
4. Yokoyama K, Carrasquillo JA, Chang AE, Colcher D, Roselli M, Sugarbaker P, Sindelar W, Reynolds JC, Perentesis P, Gansow OA, Francis B, Adams R, Finn R, Schlom J, and Larson SM, Differences in biodistribution of indium-111- and iodine-131-labeled B72.3 monoclonal antibodies in patients with colorectal cancer, Journal of Nuclear Medicine 1989;30:320-7.
5. Kumar K, Magerstadt M, and Gansow OA, Lead (II) and bismuth (III) complexes of the polyazacycloalkane-N-acetic acids nota, dota, and tete, Journal of the Chemistry Society, Chemical Communication 1989;3:145-6.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06356-06 R0

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Treatment of Malignant Brain Tumors with Interstitial Radiotherapy

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	K. Orr	Dosimetrist	ROB, NCI

## COOPERATING UNITS (if any)

Surgical Neurology Branch, NINCDS, NIH

## LAB/BRANCH

Radiation Oncology Branch

## SECTION

Radiation Therapy Section and Physics Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2

## PROFESSIONAL:

1

## OTHER:

1

## CHECK APPROPRIATE BOX(ES)

- |  |  |                                      |
|--|--|--------------------------------------|
| <input checked="" type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors                   |  |                                      |
| <input type="checkbox"/> (a2) Interviews               |  |                                      |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Median survival of high-grade gliomas remains less than a year, despite multi-modality treatment. Cure is considered anecdotal. These tumors usually have extended beyond the limits of a complete surgical resection, and the dose of conventional external beam radiotherapy has been limited by surrounding normal brain tolerance. We believe that we can achieve a higher radiation dose to the tumor by placing radioactive seeds of Iodine-125 directly into the tumor bed, with a sharp fall-off of radiation to the surrounding normal brain. Hopefully, this will achieve a much better therapeutic ratio, especially when delivered at low dose rates.

## Project Description

### Professional Personnel Engaged on Project:

D. C. Wright                      Senior Investigator                      SN, NINCDS

### Objectives

To develop a technique of interstitial implantation of intracranial tumors; to determine the acute effects and complications of such treatment; to explore the efficacy of such therapy; and to develop patient selection guidelines for future applications.

### Methods Employed

Patients with primary untreated high-grade gliomas of less than 5 cm diameter receive approximately 4000-4500 rads of external beam radiotherapy prior to interstitial implantation of radioactive Iodine-125 seeds. High-grade gliomas recurrent after prior external beam radiotherapy are treated with interstitial implantation of Iodine-125 only without additional external beam radiation. Using a Brown Robert Wells stereotactic frame and a customized template device, silastic catheters loaded with radioactive seeds of Iodine-125 are stereotactically positioned in the tumor. Catheters are then anchored to the dura, and the bone defect is closed. Catheters are left in place to deliver an appropriate radiation dose, and then are removed at the time of a second, minor surgical procedure, approximately 1 month later.

### Major Findings

Twenty-two patients have been enrolled in the protocol: 10 with primary glioblastoma multiforme, 7 with recurrent glioblastoma multiforme, 3 with primary anaplastic astrocytoma, and 2 with recurrent anaplastic astrocytoma. Two patients remain alive, 8 and 14 months after the implant procedure. Twenty patients have died 2-41 months after implant procedure. Median survival of all patients is 12.8 months after implantation. Patients with previously untreated primary glioblastoma multiforme have a median survival of 16.3 months after diagnosis, which compares favorably to reported figures in the literature for other means of treatment of primary glioblastoma multiforme, where median survival is approximately 10 months. Median survival of patients with recurrent tumors is 9.3 months after implant, which also compares favorably with reported data in the literature.

The technique for the stereotactic placement of multiple catheters containing multiple radioactive sources has been developed. Only 3 of 22 patients have required reoperation for symptomatic radiation necrosis/tumor, which compares favorably with a rate at 40% to 50% reported elsewhere in the literature. This technique can be used to implant a tumor in any cranial

site, excluding the posterior fossa which is technically inaccessible. It can be adapted to a variety of isotopes and a variety of tumor configurations.

Significance to Medical Research and the Program of the Institute

This study helps to provide information on dose and dose rate effects on both tumor and normal brain after interstitial implantation of radioactive sources.

Proposed Course

It is proposed to study a total of 30 patients. We wish to explore the development of advanced computer algorithms for radiation treatment planning and dose display. Depending on the results of treatment of the first 30 patients, a decision will be made about whether to enter into additional studies incorporating interstitial implantation of brain tumors.

Publications

None.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06357-06 R0

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Clinical Studies on Intraoperative Radiation Therapy

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: E. Glatstein Chief ROB, NCI

Others: W. Sindelar Senior Investigator SB, NCI

## COOPERATING UNITS (if any)

Surgery Branch

## LAB/BRANCH

Radiation Oncology Branch

## SECTION

Radiation Therapy Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

10

## PROFESSIONAL:

100

## OTHER

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The Radiation Oncology Branch and Surgery Branches of the National Cancer Institute have been involved in prospectively randomized studies evaluating the potential role of intraoperative radiation therapy in several disease sites, including resectable and unresectable carcinomas of the pancreas, resectable carcinomas of the stomach, and retroperitoneal sarcomas. One hundred patients have been treated with experimental intraoperative radiation therapy, and randomized to either receive or not receive radiation therapy intraoperatively with large single doses of electrons. There is really no suggestion of improvement in survival, or in disease-free survival. There is some suggestion of an improvement of local control in the retroperitoneum itself; however, this is off-set by a high predilection for seeding of the abdominal cavity, either peritoneal carcinomatosis or sarcomatosis, thus neutralizing the potential benefit of intraoperative radiation. The trials on pancreatic carcinoma and retroperitoneal sarcomas have been closed. The gastric study is still open for patient accrual.

Project Description:

## Professional Personnel Engaged on the Project:

W. Sindelar	Senior Investigator	SB, NCI
H. Pass	Senior Investigator	SB, NCI
R. Smith	Cancer Nursing Specialist	CNS, CC

Objectives: These are phase I and II studies assessing the role of intra-operative radiation therapy as an adjunct to surgical resection in various primary tumor sites, including pancreas, stomach, and retroperitoneum, where local failure following surgery alone is extremely high. Additional pilot studies are ongoing to determine the role of intraoperative radiation therapy with tumors with high-risk of local recurrence.

Methods Employed

Patients are considered for entry on the randomized studies with combined surgical resection and intraoperative therapy that have specific malignant lesions with the abdomen and retroperitoneum, and lack evidence of metastatic spread. In general, the control arm of these studies receives resection with post-operative conventional fractionated radiotherapy, and the experimental arm receives in addition, intraoperative radiation therapy, as well as misonidazole, a known radiosensitizer of hypoxic cells, a single injection of 3.5 gm/m<sup>2</sup>. Patients are followed closely to assess local toxicity, and patterns of recurrence.

Major Findings

With over 100 patients having been randomized to receive intraoperative radiation therapy at the NCI, there is no trend to suggest an improvement in local control, disease-free survival, or overall survival. Local control can be made to look quite good, if one talks only about the retroperitoneum. However, the marked predilection for carcinomatosis or sarcomatosis of the peritoneal surface itself, negates this potential gain. Until this problem can be overcome, intraoperative radiation therapy will not be useful on a large scale. Potentially, intraperitoneal chemotherapy, pre-operative radiation therapy, or intraoperative photodynamic therapy might be useful in overcoming this problem.

Significance to Biomedical Research and the Program of the Institute

Intraoperative radiation therapy studies are the first prospective randomized trials looking at this method of delivering radiation therapy.

Proposed Course

With the renovations of the electronics of the Microtron, we hope to continue



these pilot trials. However, until we are able to deal realistically with the problem of peritoneal seeding, this modality will probably not prove to be useful. If we can overcome the problem of peritoneal seeding, this may represent a useful advance in a number of abdominal neoplasms. Photodynamic approaches to prevent peritoneal seeding are presently in phase I studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 CM 06358-06 RO

PERIOD COVERED October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Radiolysis, Photolysis and Sonolysis of Cells and their Constituents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Riesz	Research Chemist	ROB, NCI
Others:	M. K. Cherukuri	Visiting Associate	ROB, NCI
	T. Kondo	Visiting Fellow	ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH Radiation Oncology Branch

SECTION Experimental Phototherapy

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS 2.5	PROFESSIONAL: 2.5	OTHER
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CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The chemical effects of ultrasound were studied in relation to hyperthermia (used in combination with radiation therapy) and the possible consequences of diagnostic and therapeutic applications. The very high temperatures and pressures induced by acoustic cavitation in collapsing gas bubbles in aqueous solutions exposed to ultrasound lead to the thermal dissociation of water vapor into H atoms and OH radicals. Their formation has been confirmed by spin trapping. Sonochemical reactions occur in the gas phase (pyrolysis reactions), the gas-liquid interface, and in the bulk of the solution (radiation chemistry reactions). The high temperature gradients in the interfacial regions lead to pyrolysis products from non-volatile solutes present at sufficiently high concentrations. The sonochemically generated radicals from carboxylic acids, amino acids, dipeptides, sugars, pyrimidine bases, nucleosides and nucleotides were identified by spin trapping with the non-volatile spin trap 3,5-dibromo-2,6-dideutero-4-nitrosobenzenesulfonate. At low concentrations of non-volatile solutes, the spin trapped radicals produced by sonolysis are due to H atom and OH radical reactions. At higher concentrations of these non-volatile solutes, sonolysis leads to the formation of additional radicals due to pyrolysis processes (typically methyl radicals). A preferred localization of non-volatile surfactants (compared to analogous non-surfactant solutes) was demonstrated by the detection of pyrolysis radicals at 500-fold lower concentrations. Pyrolysis radicals were also found in the sonolysis of aqueous solutions containing only certain nitro spin traps. The more hydrophobic the spin trap, the lower the concentration at which the pyrolysis radicals can be observed. The effect of varying the temperature of collapsing transient cavities in aqueous solutions of different rare gases and of N<sub>2</sub>O on radical yields and on cell lysis of mammalian cells was examined. The photochemistry of gilvocarcin V was investigated by electron spin resonance and spin trapping.

### Project Description

**Objectives:** The effects of ionizing and ultraviolet radiation and of ultrasound on biological macromolecules and their constituents are being investigated. Ionizing radiation damage to DNA is produced by the "direct effect" through the formation of radical ions, electrons, excited states and neutral free radicals, or by the "indirect effect" where radical species are hydrated electrons, hydrogen atoms, and hydroxyl radicals.

In the chain of events that lead to loss of biological activity, free radicals play an important role. Chemical compounds have been discovered which significantly modify radiation effects. These include: (a) electron affinity sensitizers which act on hypoxic tumor cells; (b) halogenated pyrimidines which are incorporated into DNA; and (c) cancer chemotherapy agents of the intercalating or alkylating type which sensitize tumor and normal cells. Studies of the mechanism of action of radiosensitizers and radioprotectors are necessary to design improved combinations of chemotherapy and radiation therapy.

An understanding of the mechanisms by which ionizing radiation brings about the loss of biological activity in macromolecules is likely to help in the development of new methods for altering the efficiency of cell killing with possible benefits to radiation therapy.

In the last few years, it has become apparent that superoxide anion radicals and hydroxyl radicals are found in many biological systems in the absence of either ionizing radiation or UV-photolysis. Recent reports have indicated that radicals are produced in the presence of certain anti-cancer drugs such as Bleomycin and Adriamycin. The significance of radical reactions is therefore not confined to radiation biology. It has also been shown that damage to tissues following ischemia appears to occur during reperfusion with oxygenated blood. This damage is generally considered to be due to the excessive production of superoxide radicals and hydrogen peroxide. In support of this hypothesis, it has been shown that in several model systems superoxide dismutase, catalase or allopurinol (a xanthine oxidase inhibitor) protect ischemic tissue from oxidative damage during reperfusion.

### Methods Employed

Nucleic acids, proteins and their constituents were gamma-irradiated either in the solid state or in aqueous solutions in a 800-curie Colbalt gamma-source. Electron spin resonance studies were carried out with a Varian E-9 Spectrometer connected to an IBM-XT computer. For photolysis studies at specific wavelengths, a 1000-watt high

pressure Xenon arc source and monochromator were employed. For ultrasound exposures, aqueous solutions were insulated in a non-perturbing cylindrical cell with 1 mil mylar windows in an anechoic ultrasound exposure apparatus at  $30 \pm 0.5$  degrees. Specimens were exposed to either continuous wave or tone bursts of 1 MHz ultrasound to simulate both therapeutic and diagnostic exposure conditions. In the spin trapping method, the short-lived free radicals react with a diamagnetic scavenger (the spin trap) to produce longer-lived radicals (the spin adduct) which can be conveniently investigated by e.s.r. In our studies, 2-Methyl-2-Nitrosopropane, 5,5-Dimethyl-1-Pyrroline-N-Oxide, and 3,5-dibromo-2,6-dideuterio-4-nitrosobenzenesulfonate were employed as the spin traps.

### Major Findings

I. Sonolysis of Aqueous Surfactant Solutions. Probing the Interfacial Region of Cavitation Bubbles by Spin Trapping (with A.E. Alegria, Y. Lion, and T. Kondo)

With 50 kHz ultrasound a preferred localization of non-volatile surfactants at the interface of cavitation bubbles compared to non-volatile non-surfactant solutes was demonstrated by the detection of pyrolysis-derived methyl radical spin adducts at a 500-fold lower concentration of the surfactants n-octyl- $\beta$ -D-glucopyranoside and n-decyl- $\beta$ -D-glucopyranoside than for the non-surfactant analogue methyl- $\beta$ -D-glucopyranoside.

II. Sonolysis of Concentrated Aqueous Solutions of Non-volatile Solutes: Spin Trapping Evidence for Free Radicals Formed by Pyrolysis (with T. Kondo and Murali Krishna Cherukuri)

The sonolysis of argon-saturated aqueous solutions of sodium acetate, sodium propionate, amino acids and sugars was investigated by ESR and spin trapping over a large range of concentrations. At lower concentrations of these solutes only radicals formed by hydroxyl radicals and hydrogen atom abstraction reactions could be detected. However, at the higher concentrations, new radicals (typically methyl radicals) formed in the high temperature interfacial regions induced by cavitation were formed.

III. Sonochemistry of Nitron Spin Traps in Aqueous Solutions. Evidence for Pyrolysis Radicals from Spin Traps (with T. Kondo)

Argon-saturated solutions of the spin traps a-phenyl-N-tert butylnitron, a-(4-pyridyl-1-oxide)-N-tert-butylnitron, and a-(4-nitrophenol)-N-tert-butylnitron were studied. The greater the hydrophobicity of the spin trap, as measured by the 2-octanol/water partition coefficients, the lower the concentration of spin trap at which methyl radicals generated by thermal decomposition of the spin trap can be observed. The results indicate that the non-volatile, highly hydrophobic spin traps accumulate preferentially in the interfacial regions of the cavitation bubbles where they undergo thermal decomposition during cavitation.

- IV. Hydrogen Atom Formation by Ultrasound in D<sub>2</sub>O Solutions of Nitron Spin Traps (with T. Kondo) This work was undertaken to distinguish two pathways for the formation of H atoms. The first is the thermal dissociation of water vapor in collapsing cavitation bubbles; the second is the homolytic scission of C-H bonds of the spin trap or of its decomposition products. High yields of H atoms from the spin traps were generated by pyrolysis of the spin traps when argon saturated D<sub>2</sub>O solutions of spin traps were sonicated.
- V. Pyrolysis Radicals Formed by Ultrasound in Aqueous Solutions of Nucleotides. A Spin Trapping Study. (with T. Kondo and C. Murali Krishna) For a better understanding of the degradation of DNA exposed to ultrasound, knowledge of the sonochemistry of nucleotides is desirable. At low nucleotide (0.05M) the spin trapped radicals produced by sonolysis are due to H atom and OH radical reactions, typically addition to the 5,6 double bond of the base moiety. At high concentrations (1.0 M) pyrolysis radicals were found. The results indicate that pyrolysis radicals can be detected when nucleotides are accumulated at high concentrations in the interfacial regions of cavitation bubbles.
- VI. Sonochemistry of Alcohol-Water Mixtures. Spin Trapping Evidence for Thermal Decompositions and Isotope Exchange Reactions (with C. Murali Krishna, and T. Kondo) Free radical intermediates induced by 50 kHz ultrasound in aqueous solutions of ethanol, 1-propanol, 2-propanol, and tert. butyl alcohol were identified. In the sonolysis of the mixed isotope systems of the type ROD:D<sub>2</sub>O and CH<sub>3</sub>CD<sub>2</sub>OH:H<sub>2</sub>O, isotopically mixed radicals were spin trapped indicating the occurrence of multiple radical recombination reactions in the gas phase of collapsing cavitation bubbles. The effect of temperature on the sonochemical yield of 10% ethanol was studied and was found to decrease with increasing temperature in the range 25-50° C, indicating the predominant effect of vapor pressure of the bulk solvent on the sonochemical yields.
- VII. An ESR Study of the Visible Light Photochemistry of Gilvocarcin V. (with A.E. Alegria, C. Murali Krishna and R.K. Elespuru (Lab. of Chemical and Physical Carcinogenesis, LBI-Basic Research Program, NCI, Frederick) Photolysis of gilvocarcin (GV) at 405 nm in argon-saturated dimethyl-sulfoxide (DMSO) leads to the formation of methyl radicals by photoreduction of DMSO by GV. GV also photoreduces oxygen and methylviologen with quantum yields of 0.019 and 0.0012, respectively. The quantum yield for singlet oxygen formation by GV in DMSO was found to be 0.15. Hence, both Type I and Type II pathways could contribute to the phototoxicity of GV in biological systems.
- VIII. A Novel Metal-free Low Molecular Weight Superoxide Dismutase Mimic (with A. Samuni, C. Murali Krishna, E. Finkelstein and A. Russo) Oxazolidine nitroxides were shown to act as superoxide dismutase mimics. Since these compounds exhibit cell permeability and relative stability they might be used as superoxide dismutase mimics inside and outside of cells.

- IX. Free Radicals Induced by Adriamycin-Sensitive and Resistant Cells: A Spin Trapping Study. (with A.E. Alegria, A. Samuni, J.B. Mitchell and A. Russo)  
The effect of adriamycin on the radicals generated by adriamycin sensitive (CHO-AB) and resistant (CHO-C5) Chinese hamster ovary cells as well as adriamycin-sensitive and resistant human breast cancer cells (MCF7-WT and MCF7-ADR) was studied. Since ADR-resistant and sensitive cell lines produced comparable levels of semiquinone and oxygen radicals, there is little support for the assumption that ESR-observable oxygen-derived radicals play a role in adriamycin antitumoral activity.

#### Significance to Biomedical Research and the Program of the Institute

Studies of the effects of ionizing radiation are of importance in relation to (1) radiation therapy; (2) carcinogenesis; (3) stability of the genetic pool; (4) the suppression of the immune mechanism; and (5) aging. The effects of ionizing radiation on nucleic acids are being studied in order to understand the nature of radiobiological death in normal cells, and tumor cells. The addition of radioprotective and radiosensitizing agents is being investigated so that a therapeutic advantage may be gained.

#### Proposed Course

To continue studies on the effects of ionizing radiation on mammalian cells and macromolecules of biological importance. The mechanism of radioprotective and radiosensitizing agents and the interaction of radiation and cancer chemotherapy agents will be investigated. New areas of interest include photosensitized cell killing by porphyrins and phthalocyanines in relation to photodynamic therapy and chemical and biological effects of ultrasound.

#### Publications

1. Alegria AE, Riesz P. Photochemistry of aqueous adriamycin and daunomycin. A spin trapping study with  $^{17}\text{O}$ -enriched oxygen and water, Photochem Photobiol 1988;48:147-52.
2. Kondo T, Murali Krishna C, Riesz P. Effect of non-volatile scavengers of hydroxyl radicals on the thymine radical formation induced by gamma-rays and ultrasound, Int J Radiat Biol 1988;53:891-9.
3. Kondo T, Murali Krishna C, Riesz P. Sonochemistry of nucleic acid constituents in aqueous solution. A spin trapping study. In: Simic MG, Taylor KA, Ward JF, Von Sonntag C, eds. Oxygen radicals in biology and medicine. New York: Plenum Press, 1988;433-6.
4. Kondo T, Murali Krishna C, Riesz P. Sonolysis, radiolysis, and hydrogen peroxide photolysis of pyrimidine derivatives in aqueous solutions. A spin trapping study, Radiat Res 1988;116:56-73.

5. Alegria AE, Cox O, Duman J, Rivers LA, Riesz P. Photochemistry of aqueous solutions of quinolinium salts. A spin trapping study using  $^{17}\text{O}$  enriched water and oxygen, *Biochem Biophys Acta* 1988;967:1-10.
6. Kondo T, Gamson J, Mitchell JB, Riesz P. Free radical formation and cell lysis induced by ultrasound in the presence of different rare gases, *Int J Radiat Biol* 1988;54:954-62.
7. Samuni A, Murali Krishna C, Riesz P, Finkelstein E, Russo A. A novel metal-free low molecular weight superoxide dismutase mimic, *J Biol Chem* 1988;263:17921-4.
8. Samuni A, Murali Krishna C, Riesz P, Finkelstein E, Russo, A. Superoxide reaction with nitroxide spin-adducts, *Free Radical in Biol and Med* 1989;6:144-8.
9. Alegria AE, Murali Krishna C, Elespuru PK, Riesz P. An ESR study of the visible light photochemistry of Gilvocarcin V, *Photochem Photobiol* 1989;49:257-65.
10. Kondo T, Fukushima Y, Kon H, Riesz P. Effect of shear stress and free radicals induced by ultrasound on erythrocytes, *Arch Biochem Biophys* 1989;269:381-9.
11. Kondo T, Riesz P. Sonolysis of concentrated aqueous solutions of nonvolatile solutes. Spin trapping evidence for free radicals formed by pyrolysis, *Radiat Res* 1989;118:211-29.
12. Kondo T, Riesz P. Sonochemistry of nitron spin traps in aqueous solutions. Evidence for pyrolysis radicals from spin traps, *Free Radicals in Biol and Med* 1989 (in press).
13. Murali Krishna C, Kondo T, Riesz P. Sonochemistry of alcohol-water mixtures. Spin trapping evidence for thermal decomposition and isotope exchange reactions, *J Phys Chem* 1989 (in press).
14. Alegria AE, Lion Y, Kondo T, Riesz P. Sonolysis of surfactants in aqueous solutions. Probing the interfacial region of cavitation bubbles by spin trapping, *J Phys Chem* 1989 (in press).
15. Kondo T, Riesz P. Hydrogen atom formation by ultrasound in  $\text{D}_2\text{O}$  solutions of nitron spin traps, *Free Radical Res Comm* 1989 (in press).
16. Kondo T, Murali Krishna C, Riesz P. Pyrolysis radicals formed by ultrasound in aqueous solutions of nucleotides. A spin trapping study, *Int J Radiat Biol* 1989 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 CM 06360-06 R0
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Radionuclide Generators to Produce the Iridium-194 Beta Emitter</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <b>PI: Saed Mirzadeh Expert ROB, NCI</b>  <b>Others: Otto A. Gansow Senior Investigator ROB, NCI</b>		
COOPERATING UNITS (if any)		
LAB/BRANCH Radiation Oncology Branch		
SECTION Inorganic and Radioimmune Chemistry Section		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The project investigates the design, manufacturing, testing, and use of a novel radionuclide generator for biomedical applications. The generator has a long shelf-half-life, many years, and produces a 20-hour radionuclide daughter which emits high-energy beta particles that have suitable characteristics for labelling proteins through bifunctional chelates.</p> <p>In the 194-Os/194-Ir generator containing the parent nuclei, 194-Os, has a half-life of 6.0 years which beta-decays, <math>E_{max} = 100</math> KeV, to the 19.15-hour 194-Ir daughter. The 194-Ir daughter decays with the emission of 2.2 MeV beta particles to the ground state of 194-Pt (86%) and to the 328.5 KeV first excited state with emission of 1.9 MeV beta particles (9.2%). There is a 328.5 KeV gamma-ray which follows the decay of 194-Ir with 13% absolute abundance. The absence of high intensity gamma-rays in the decay of 194-Ir, with the exception to the 328.5 KeV, makes this beta emitter nuclei very attractive from the point of view of dosimetric considerations. On the other hand, the presence of 328.5 KeV gamma-rays makes 194-Ir a superior nuclei to 90-Y for tumor imaging.</p> <p>Preliminary calculations indicate that several mCi of the parent, 194-Os, can be produced in a nuclear reactor by double neutron capture of an 192-Os (natural abundance of 41%) target. By using enriched 192-Os, a two-fold increase in the yield results and also reduces the production of impurities. The enriched Osmium-192 with enrichment factor of greater than 99% is purchased from Oak Ridge National Laboratory.</p>		



### Project Description

Objective: To develop a radionuclide generator system which will produce  $^{194}\text{Ir}$  for attachment to proteins through bifunctional chelations.

### Methods Employed

Osmium- $^{194}$  is produced in a nuclear reactor by irradiating enriched Os- $^{192}$ . After irradiation, Os target is dissolved, purified and loaded into a suitable chromatographic column. The daughter  $^{194}\text{Ir}$  is eluted with suitable solvents. The yield of the elution of the daughter, the breakthrough of the parent, radiation and chemical resistance of the column generator, radiochemical, and radionuclidic purity of the product are being investigated.

In addition, to test the integrity of the quartz ampule target containers in high radiation field, a series of preliminary irradiation were performed at the Brookhaven National Laboratory High Flux Beam Reactor (BNL-HFBR). Several blank quartz ampules were irradiated at the core of the reactor for 21 days (duration of the reactor cycle). No chemical fatigue so far has been observed.

In November 1988, 10 mg of enriched Os- $^{192}$  (99.395%) was irradiated at BNL HFBR for a period of 21 days (a full reactor cycle), at position "Modified V16" with thermal neutron flux of  $8.25 \times 10^{14}/\text{sec. cm}^2$ . The preliminary results indicate that a 20-mCi generator can be produced by irradiating 100 mg of enriched Os- $^{192}$  for a period of 3 months (3 reactor cycles). Presently, the decay of Os- $^{194}$  is followed to obtain an accurate measure of its production cross-section. With the recent preparation of the bi-capped trencam ligands now in use for Gallium (III), bifunctional chelating agent for Ir- $^{194}$  is available in our group.

### Significance to Biomedical Research and the Program of the Institute

The development of the  $^{194}\text{Os}/^{194}\text{Ir}$  generator for production of 20-hour  $^{194}\text{Ir}$  would increase access of the biomedical community to a high-energy beta emitter as a radiotherapeutic agent.

### Proposed Course

- 1) Development of  $^{194}\text{Os}/^{194}\text{Ir}$  generator.
- 2) Prepare suitable bifunctional chelates for attachment of  $^{194}\text{Ir}$  for proteins.
- 3) Study proteins labeled with  $^{194}\text{Ir}$  in vitro and in vivo.

### Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 CM 06361-05 RO</b>
PERIOD COVERED <b>October 1, 1988 to September 30, 1989</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Phototherapy of Intracavitary Spaces</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <b>PI: A. Russo</b> </div> <div style="width: 30%;"> <b>Senior Investigator</b> </div> <div style="width: 30%;"> <b>ROB, NCI</b> </div> </div> <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <b>Others:</b>            J. B. Mitchell            H. Pass            P. Smith            W. Frauf            C. Black            E. Bernstein         </div> <div style="width: 30%;"> <b>Senior Investigator</b>  <b>Senior Investigator</b>  <b>Senior Investigator</b>  <b>Senior Investigator</b>  <b>Associate</b>  <b>Associate</b> </div> <div style="width: 30%;"> <b>ROB, NCI</b>  <b>SOB, NCI</b>  <b>BEIB</b>  <b>BEIB</b>  <b>ROB, NCI</b>  <b>ROB, NCI</b> </div> </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH <b>Radiation Oncology Branch</b>		
SECTION <b>Experimental Phototherapy Section</b>		
INSTITUTE AND LOCATION <b>NCI NIH, Bethesda, Maryland 20892</b>		
TOTAL MAN-YEARS <div style="border: 1px solid black; text-align: center; width: 40px; margin: 0 auto;">7</div>	PROFESSIONAL <div style="border: 1px solid black; text-align: center; width: 40px; margin: 0 auto;">7</div>	OTHER <div style="border: 1px solid black; text-align: center; width: 40px; margin: 0 auto;">0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             The use of hematoporphyrin derivative and other photosensitizing agents in combination with light activation is currently being investigated as an anti-tumor modality for the treatment of intraperitoneal and intrathoracic tumors. A major advantage of this modality is the apparent selective retention of the sensitizing dye within tumors. A murine ascites ovarian carcinoma and a human ovarian tumor that grows as an ascites tumor has been used to study the characteristics of drug distribution in the peritoneal cavity. Likewise, murine models have been used to study the tolerance of the thoracic cavity structures to the phototherapy techniques being explored. The limitations of the murine model has required the extensions of the investigation to the canine model for evaluation of the toxicity of Phototherapy. Different wavelengths of light, different laser delivery systems, different sensitizers, different doses of energy, different modes of drug administration, and different monitoring devices are being studied. We have shown that Phototherapy can be used to effectively treat a murine ascites tumor. We have also shown that in both the murine and the canine model, the peritoneal serosal surface is tolerant of at least 0.5 J/cm<sup>2</sup> and that this work can be extended to human subjects. In the murine system, we have shown that the thoracic cavity, like the peritoneal cavity, is exquisitely sensitive to treatment with red light (630 nm). The dose rate must be controlled to minimize heat build up (less than 150 mW fiber output from a forward projecting optical fiber is usually tolerated). We are exploring the use of photoimmunotherapy as an additional means of drug delivery. We are exploring the use of chemiluminescence as a means of light delivery to the cavity spaces.           </p>		

### Project Description

**Objective:** To establish a laboratory model for treatment of intracavitary malignancies that spread by implanting on the serosal surfaces of organ such that Hematoporphyrin derivative and other photosensitizing agents can be used in combination with non-ionizing radiation. To determine the best means of delivering light and sensitizer and to establish means to better quantitate light delivered to the tumor and normal tissue (dosimetry), to originate means of improving or circumventing phototoxicity to normal tissue, and to provide means to remove viruses outside of cells or cells containing viruses either inside the cell or incorporated into the human DNA in banked blood intended for human use are secondary objectives of the study.

### Methods Employed

Two different murine (thymic and athymic) systems and a canine model are being used to investigate the peritoneum for Phototherapy. For the study of the chest cavity, murine and canine models are being studied. Response, survival, histopathology are used for evaluation. In vitro cell culture techniques are being used to judge the initial effects of different sensitizers. Both pleiotropic drug resistant cell systems as well as more conventional cell models are being used. Fluorescence spectroscopy is being used to study drug administration routes as they impact on tumor localization and normal tissue distribution. Light dosimetry is being studied by photodiode placement and computer modeling and analysis. Monoclonal antibodies are being affixed to either Porphyrin C or hexaethylmethylporphyrine-3-propionic acid because the sensitizers can be purified to homogeneity, have desirable absorbance characteristics, and provide different chemical means of attachment. Antibodies being studied are directed against either human lung or ovarian tumors that have been developed for growth in an athymic murine model system. General searches for sensitizers that absorb light at longer wavelengths (>600 nm) are being sought that also have the characteristics of being lipid membrane permeable and favorably partition to nucleic acid oligomers. Such sensitizers are investigated for viricidal effect.

### Major Findings

For a murine model of an ovarian ascites tumor, phototherapy is effective (85% long-term survival) when using HPD and green light. Red light cannot be used in a murine model of intra-cavitary treatment because the dye is retained in liver and red light can penetrate the small size of a murine liver. Dogs tolerate one, two, and three treatments of intraperitoneal light therapy after iv and ip administration of HPD. Dose rate and total dose of light impact on tumor response. Initial work in a cell culture system that has been pretreated with HPD shows that chemiluminescence agents provide enough light to be effectively used as a light source. Plastic models of a canine thoracic cavity suggest that intralipid (fat emulsion) can be used for real-time simultaneous equal light distribution to the pleural surface when three or more fiber sources are concurrently used. Pleiotropic drug resistant CHO cells (C5) are slightly more resistant than the wild type. The resistance correlates with the decrease in cellular concentration of sensitizer. Pleiotropic drug resistant human mammary carcinoma cells are no more resistant than the corresponding wild type. HTLV-III viral particles can be rendered non-infectious by HPD and light. Histamine antagonist, both H-1 and H-2, can be used to decrease the dermal photosensitivity caused by HPD.

Significance to Biomedical Research and the Program of the Institute

The ROB is involved in clinical use of Phototherapy and this work is being applied to guide choice of tumors to treated, the dosing of light to be used, and the best means of administering sensitizer.

Proposed Course

Continue to explore the models outlined above to improve the use of Phototherapy in the clinic.

Publications

1. Russo A, Mitchell JB, Pass HI, Glatstein, E. Photodynamic therapy. In: DeVita V, Hellman S, Rosenberg S, eds. Principle and practice of oncology. Philadelphia: JB Lippincott, 1989;2449-61.
2. Manyak MJ, Smith PD, Harrington FS, Steinberg SM, Glatstein E, Russo A. Protection against dihematoporphyrin ether photosensitivity. Photochem Photobiol 1988;47(suppl 6):823-30.
3. Manyak MJ, Matthews DM, Smith PD, Nochomovitz LE, Russo A. Photodynamic therapy: response of normal canine urethra using a cylindrical fiber. Lasers Surg Med 1988;8(suppl 3):301-7.
4. Manyak MJ, Russo A, Smith PD, Glatstein E. Photodynamic therapy. review article: 116 Refs. J Clin Oncol 1988;6(suppl 2):380-91.
5. Manyak MJ, Matthews DM, Smith PD, Nochomovitz LE, Glatstein E, Russo A. Response of normal canine ureter to photodynamic therapy using a cylindrical fiber. J Urol 1988;139(suppl 1):199-203.
6. Russo A, Mitchell JB. Future directions of photodynamic therapy, In: Phototherapy of human tumors, (in press).
7. Mitchell JB, Cook JA, Russo A. Biological basis for phototherapy. In: Phototherapy of human tumors, (in press).

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <div style="border: 1px solid black; padding: 2px; text-align: center;">Z01 CM 06363-06 RO</div>
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>DNA Damage by Alkylating Agents and Their Repair in Human Tumor Cells</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between;"> <span>PI: A. J. Fornace, Jr.</span> <span>Senior Investigator</span> <span>ROB, NCI</span> </div>		
COOPERATING UNITS (if any) Applied Genetics, Freeport, New York (D. Yarosh).		
LAB/BRANCH Radiation Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: .02	PROFESSIONAL: .02	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div> <input type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <div style="border: 1px solid black; padding: 10px; min-height: 200px;"> <p>R. S. Day, III, D. Yarosh, and others have shown that approximately 20% of human tumor lines and viral transformed lines are hypersensitive to alkylating agents due to an apparent absence of alkylguanine alkyltransferase. This enzyme removes alkylation damage at the 0-6 position of guanine but not at other sites in DNA. Dr. D. Yarosh in collaboration with our unit has been able to partially purify the enzyme from human liver and raise polyclonal antibodies to this protein. Studies have been initiated to develop monoclonal antibodies to this protein. With high affinity antibodies, we will be able to further purify this protein. With sufficient purification, partial amino acid sequence can be obtained and used to synthesize oligonucleotide probes which will be used to screen human liver cDNA libraries. Such antibodies can also be used to screen human liver cDNA expression libraries.</p> </div>		

Project Description

Objective: To purify the human alkylguanine alkyltransferase enzyme and ultimately isolate the gene.

Methods Employed

Standard molecular biology and protein biochemistry approaches.

Major Findings

Polyclonal antibodies to this protein have been isolated, and efforts are directed at isolating high affinity polyclonal and monoclonal antibodies. The primary effort is being made by D. Yarosh to isolate high affinity antibodies to this elusive protein.

Significance to Biomedical Research and the Program of the Institute

An understanding of this defect which occurs in approximately 20% of all human tumor lines would have obvious importance in both carcinogenesis and cancer treatment.

Proposed Course

When high affinity antibodies have been developed, efforts will be made to isolate cDNA clones for this protein using expression libraries.

Publications

None.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <div style="text-align: center;">Z01 CM 06365-06 RO</div>
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>cDNA Cloning and Characterization of Genes Induced by Hyperthermia</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	A. J. Fornace, Jr.	Senior Investigator ROB, NCI
Others:	I. Alamo M. C. Hollander	Microbiologist Microbiologist ROB, NCI ROB, NCI
COOPERATING UNITS (if any)		
LAB/BRANCH Radiation Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 1.0	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided )		
<p>Prokaryotic and eukaryotic cells respond to environmental stress by the induction of a variety of stress-related proteins. In mammalian cells, the most well-characterized group of stress proteins are induced by hyperthermia. Transcription of heat shock proteins increases markedly after hyperthermia, and several of these genes have been cloned from human and mouse cells in other laboratories. It is likely that transcription of other genes is also induced in mammalian cells since approximately 10-20 genes are induced in prokaryotes and lower eukaryotes. We have isolated cDNA clones coding for more than 15 different heat shock-induced RNA in Chinese hamster cells by differential hybridization screening of a cDNA library which we constructed from heat shock-treated cells. Based on homology to human heat shock cDNA clones, some of our cDNA clones were found to code for hsp70, hsp27, hsp60, hsp89<math>\alpha</math>, and hsp89<math>\beta</math>. Except for hsp60, all these genes were coordinately induced by heat shock. By DNA sequencing the 2 most abundant isolates were found to code for ubiquitin and B2 RNA polymerase III transcripts. Ubiquitin plays many important roles in eukaryotic cells, including a prominent role in the removal of damaged proteins. Ubiquitin has been found to be induced by heat shock in yeast and chicken cells, but ours is the first demonstration in mammalian cells. We estimated that up to 10% of the mRNA in heat shock-treated cells were ubiquitin transcripts. We have found also that ubiquitin transcription was strongly induced in both rodent and human cells by alkylating agents which indicates a possible role for ubiquitin in the cellular response to such damage; in yeast, the RAD6 DNA repair gene is a ubiquitin-conjugating enzyme. These studies have been extended to other heat shock genes and we have found that hsp27, in particular, was induced by certain DNA-damaging agents. In the case of B2 RNA, we constructed size-selected libraries from untreated and heat shock-treated cells, and isolated 200 B2 cDNA clones. By DNA sequencing, the heat shock-inducible B2 transcripts consistently differed from those of unheated cells.</p>		

### Project Description

Objective: To identify genes induced by hyperthermia in Chinese hamster cells, and to study their regulation.

### Methods Employed

Standard molecular biology approaches and hybridization subtraction.

### Major Findings

We have isolated cDNA clones coding for many of the transcripts induced by heat shock in Chinese hamster cells. These genes were coordinately regulated by heat shock. Maximum induction of transcription of these genes correlated with the induction of thermotolerance (cellular resistance to cytotoxicity of hyperthermia). Some were induced also by DNA-damaging agents which indicates a possible role for certain heat shock proteins in genotoxic stress. We have demonstrated for the first time in mammalian cells that ubiquitin RNA is a major heat shock-induced transcript. We have found that RNA polymerase III transcription of the B2 repetitive genetic element was induced by heat shock. By sequence analysis, the B2 genes induced by heat shock differed from those constitutively expressed. We have initiated studies with thermotolerant heat-sensitive mutants, and have found that the regulation of hsp mRNA, particularly ubiquitin and hsp70, is altered in a particular Chinese hamster cell line.

### Significance to Biomedical Research and the Program of the Institute

A more thorough understanding of the response of mammalian cells to hyperthermia would benefit both clinical hyperthermia research and also how mammalian cells respond to environmental stress. In the field of experimental hyperthermia, much of the cell biology has been with Chinese hamster cells; our cDNA clones can now be used to study these events at the molecular level.

### Proposed Course

The response in mutant cells with altered sensitivity to hyperthermia will be studied. Study of the induction of these genes after different types of stress, such as oxidative stress, will be continued.

### Publications

1. Fornace AJ Jr, Alamo I Jr, Hollander MC, Lamoreaux E. Ubiquitin mRNA is a major stress-induced transcript in mammalian cells, *Nucleic Acids Res* 1989;17:1215-30.
2. Fornace AJ Jr, Alamo I Jr, Hollander MC. Induction of heat shock protein transcripts and B2 transcripts by various stresses in Chinese hamster cell, *Experimental Cell Res* 1989;182:61-74.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06369-06 R0

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

Radiation Characteristics of the Scanditronix MM-22 Medical Microtron

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)

PI: R. Miller Radiation Physicist ROB, NCI

Others: J. van de Geijn Radiation Physicist ROB, NCI  
B. Chin Arora Clinical Physicist ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.5

PROFESSIONAL

2.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )

The Physics Section is continuing studies of the radiation characteristics of the Scanditronix MM-22 medical Microtron. Current research is centered around our Intraoperative Radiotherapy Program (IORT) and concerns the dosimetry of special IORT applicators. Associated with this is a study of the effects of using asymmetric electron collimation on the dose distributions of these applicators. A new color camera television system for viewing the intraoperative portal is under development. This system will employ a permanent mirror of aluminized Mylar instead of the current retractable glass mirror. This should allow remote viewing of the radiation portal during treatment.

### Project Description

Objectives: The optimization of the radiation and operational characteristics of the Scanditronix MM-22 Microtron.

### Methods Employed

The high quality and versatile radiation measurement systems available to the Branch are used by staff personnel in cooperation with experts from the manufacturer to determine the basic performance of the various critical functions of the machine and its monitoring equipment. Several of these functions have been found less than optimal for the special purposes envisioned by the ROB and in several cases, dramatic improvements have been obtained already.

### Major Findings

Several performance characteristics were unsatisfactory for our purposes: electron depth dose distribution and transverse beam profiles, monitor characteristics. Interaction between representatives of our staff and experts from the firm have resulted already in performance characteristics much better than required by the specifications.

### Significance to Biomedical Research and the Program of the Institute

The Microtron is to be utilized primarily for intraoperative radiotherapy. As such, its reliability and (especially) its beam characteristics are of critical importance to this program. Also, the machine offers some technical features such as independently adjustable collimator jaws, which are meaningful only with more than minimum performance characteristics.

### Proposed Course

The Microtron has recently been repaired and recommissioned. Studies are continuing at a temporarily reduced scale due to staff shortages.

### Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06370-05 RO

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Optimization of Treatment Planning for Brain Implants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. van de Geijn Radiation Physicist ROB, NCI

Others: R. Miller Radiation Physicist ROB, NCI  
E. Lamoreaux Computer Specialist ROB, NCI  
H. Xie Computer Specialist ROB, NCI  
K. Yeakel-Orr Dosimetrist ROB, NCI  
F. Harrington Biomed. Engineering Tech. ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2

PROFESSIONAL

1

OTHER

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided)

This essentially is a continuing project, guided by the continuing experience of the medical principals and the continuing development especially in computing. The purpose of this work is to optimize physics, computer based and technical support for a brain implant protocol. In particular, it aims at optimization of the chain of procedures comprising patient data acquisition, treatment planning, including optimal delineation of the target, determination of the number of radioactive sources, their strength and position, and determination of the surgical mechanical positioning devices and the further development and adaptation of locally developed computer programs, with emphasis on versatile imaging as a basis for optimization of irregular implants.

Project Description

## Personnel:

T. DeLaney	Radiotherapist	ROB, NCI
D. Wright	Neuro-Surgeon	SN, NINCDS

Objective: To develop a computer-assisted system for optimization of the physical and technical aspects of radioactive seed implants in brain tumors; criteria are:

1. Accurate fitting of a critical dose rate surface around the chosen target volume, which may be a regular or irregular shape.
2. A uniform dose distribution inside the target.
3. A minimum number of catheters positioned in the brain in the most economical and accurate way.

Methods Employed

1. Mathematical/physical methods are employed to develop a generalized approach to seed placement and relative seed strength. The effort now concentrates on irregularly shaped tumors.
2. Computer-based image manipulation of diagnostic CT data to determine optimal access routes. Current developmental efforts are directed toward implementation on a Mac II.
3. Development of mechanical positioning and directioning devices for use in data acquisition and in surgery.

Major Findings

Major results have been reached in the mathematical optimization, which is partly for practical clinical reasons, in part computerized, in part manual-based, so that a semi-automatic interactive computer based system will shortly be available. Considerable work needs to be done to make the system universal. An atlas is available for regular-shaped implants.

Significance to Biomedical Research and the Program of the Institute

The system is essential to technically and physically safe and radiation-economic patient treatment. The development aims at general applicability. The theoretical and computer related procedures apply to implants in general.

Proposed Course

Continuation of current research. A new brachytherapy program using an Apple Macintosh II computer has been started. This will provide enhanced three dimensional viewing capabilities and will utilize information from CT and MRI. Existing dose calculational models will be united and extended to provide other types of intensity distributions from implanted sources for use with hyperthermia systems and photodynamic therapy.

Publications

None.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 CM 06374-05 RO
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effect of Radiosensitizers and Radioprotectors on DNA Damage Produced by X-rays		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between;"> <span>PI: A. J. Fornace, Jr.</span> <span>Senior Investigator</span> <span>ROB, NCI</span> </div>		
COOPERATING UNITS (if any)  University of Wisconsin, Madison, WI (T. Kinsella).		
LAB/BRANCH Radiation Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: .02	PROFESSIONAL: .02	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             We have completed studies with the halogenated pyrimidine radiosensitizers, IUdR and BUdR. IUdR is currently in clinical trials as a radiosensitizer. This agent sensitizes cells to radiation after incorporation into cellular DNA in place of thymidine. We have previously shown that the yield of DNA strand breaks was increased in cells containing IUdR after x-irradiation. Current models of IUdR and BUdR radiolysis predict that only single strand damage should be produced in DNA. However, the lesion most important in x-ray lethality is probably DNA double strand breaks. We have found that radiolysis of IUdR in DNA of cells x-irradiated leads to mobile reactive intermediates which damage both the strand containing the IUdR and also the complementary strand which did not contain IUdR in these experiments. IUdR-induced strand breaks were almost as frequent in the unsubstituted strand as in the substituted strand. There was also a smaller increase in strand breaks in unsubstituted duplex DNA in cells containing IUdR-DNA. In cell survival experiments, cells undergoing only 1 doubling with IUdR showed almost as much radiosensitization as cells undergoing 2 doublings where both strands were substituted.           </p>		

### Project Description

**Objective:** To study the effect of radioprotectors and radiosensitizers on particular types of DNA damage.

### Methods Employed

Alkaline and neutral elution. Standard cell culture techniques.

### Major Findings

Radiolysis of DNA containing halogenated pyrimidines leads to the generation of mobile reactive intermediates which can produce double strand DNA damage, in particular double strand breaks.

### Significance to Biomedical Research and the Program of the Institute

There are 2 major implications for radiotherapy:

1. Significant radiosensitization can be achieved in cells undergoing only 1 round of replication in halogenated pyrimidines. Therefore, efforts should be directed toward labelling as high a fraction of the tumor cells as possible, even if many have only been labelled on 1 DNA strand.
2. DNA double strand breaks are probably responsible for much of X-ray cell lethality. We infer from our studies that the use of maximum achievable concentrations of IUdR may be preferable, since it will produce the greatest density of intermolecular damage (double strand breaks) with radiation.

### Proposed Course

A manuscript has been prepared for submission for publication. In collaboration with T. Kinsella, studies have been continued on the incorporation of IUdR into mammalian cells with particular emphasis on the effect of unifilar substitution at different levels on radiation sensitivity.

### Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06377-04 RO

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Optimization of Dose Distributions from Intraoperative Applicators

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. van de Geijn Radiation Physicist ROB, NCI

Others: R. Miller Radiation Physicist ROB, NCI  
K. Yeakel-Orr Dosimetrist ROB, NCI  
F. Harrington Biomed. Engineering Tech. ROB, NCI  
T. DeLaney Senior Investigator ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.5

PROFESSIONAL:

2

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Due to the high single fraction doses employed in intraoperative radiotherapy, it is important to minimize areas of high dose. These areas arise due to the scattering relationship between the photon collimators and the applicator system. They may be non-symmetric due to either an asymmetric applicator design, or to manufacturing tolerances of the applicator system resulting in the center of the applicator to be slightly different than the central ray of the accelerator. These dose distributions can be optimized to predetermined criteria by the use of an optimum field size for each combination of applicator and electron energy. The use of asymmetric collimators can further correct for nonuniformity in the dose distribution due to asymmetric design, applicator bevel angle, or applicator mis-alignment.



## Project Description

Objective: To optimize the dose distribution from applicators used to deliver intraoperative radiation therapy with high energy electron beams.

## Methods Employed

1. To develop criteria for defining the optimum dose distribution.
2. To study the dose distributions from applicators as a function of collimator setting and energy in order to determine optimum field sizes for each energy.
3. To use asymmetric collimators settings to correct for non-uniformities in the radiation field due to applicator shape, non-concentric applicator position and applicator bevel angle.

## Major Findings

The criteria for defining the optimum dose distribution based on the prescription isodose line have been developed. These have been used to determine optimum field sizes for the applicators currently used to deliver IORT at the NCI. The areas of high dose have been reduced by as much as 25% by this method. The use of decoupled collimators to produce asymmetric fields can restore symmetry to the dose distribution when it is affected by applicator shape or non-concentric position.

## Significance to Biomedical Research and the Program of the Institute

Most institutions performing intraoperative radiotherapy use a fixed collimator setting for each applicator regardless of energy. Some places use the same field size for all applicators as well. This clearly results in areas of high dose in the treatment which can result in doses 30%-40% greater than the prescription dose. Proper choice of field size can substantially reduce these "hot spots" resulting in a more uniform dose distribution.

## Proposed Course

Now that the MM-22 Microtron has been repaired and re-accepted, this project has been reactivated and prioritized relative to the current status of the clinical program in intraoperative radiotherapy (IORT).

## Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06378-04 RO

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

QA of Treatment Delivery by Means of Overlaid Digitized Simulator & Port Films

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. van de Geijn Radiation Physicist ROB, NCI

Others: R. Miller Radiation Physicist ROB, NCI  
B. Chin Arora Radiation Physicist ROB, NCI  
H. Xie Computer Specialist ROB, NCI  
E. Lamoreaux Computer Specialist ROB, NCI  
K. Yeakel-Orr Dosimetrist ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2

PROFESSIONAL:

.25

OTHER:

1.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The quality assurance of the consistency of radiation treatment delivery with the prescription is a continual concern, locally as well as nationally. The ROB already employs gratitudes projecting onto all simulator films and all corresponding portfilms. A project has been started to overlay differently processed digitized films to increase the quality of information, as well as to decrease the volume of documentation to be retained.

The system should be of great interest to inter-institutional studies as well. The project has been on hold until recently because of delays in acquisition of essential hardware. It is progressing rapidly now.

### Project Description

- Objective: 1) To improve the quality of documentation on the proper implementation of beam treatment set-ups.
- 2) To condense the amount of documentation to be kept, and to increase its objectivity and exchangeability.

### Methods Employed

1. Take x-ray films at the simulator, in the planned beam positions, including gratitudes projected onto the films.
2. Follow similar procedure at the treatment machine, producing port films with gratitudes.
3. Digitize both categories of films taking care to use the same orientation, centering and magnification, with help of the gratitudes projected onto all films.
4. Apply appropriate computer enhancement of both simulator films and the corresponding port films.
5. Overlay technique, bring out salient anatomical features, gratitudes, block delineation, etc.
6. Using computer, do measurements of significant deviations.
7. Store the results, properly labeled.

### Major Findings

Great progress has been made over the last decades in diagnostic imaging and computerized treatment planning. The overall quality assurance, control and documentation of actual treatment delivery is not at the same level. This project promises major improvement.

### Significance to Biomedical Research and the Program of the Institute

1. Quality assurance and verification will become much more efficient, self-contained and attractive to use.
2. Documentation will be much more compact and easier to use.
3. Quality assurance of joint studies will be much easier and more objective.

### Proposed Course

To implement the system in the Macintosh II environment and start a pilot project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 CM 06379-03 R0

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phase I Study of Photodynamic Therapy for Surface Malignancies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	E. Glatstein	Branch Chief	ROB, NCI
	A. Russo	Senior Investigator	ROB, NCI
	L. Dachowski	Nursing Clinician	ROB, NCI
	G. Thomas	Microbiologist	ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Photodynamic therapy involves the use of a light activated compound which localizes in tumor, followed by the activation of this compound by light for cytotoxic effects for the treatment of cancer. The current protocol uses the intravenous administration of the Photofrin II preparation of the hematoporphyrin derivative, the only currently approved photosensitizer for use in humans. This is followed by the delivery of light to the affected area using optical fibers coupled to an argon/pumped dye laser. Hematoporphyrin derivative selectively localizes in tumor compared to certain normal tissues. Selective retention of the photosensitizer in combination with focal light delivery to the involved area permits selective destruction of tumor with minimal effect on uninvolved normal tissue. Hematoporphyrin derivative photodynamic therapy may be clinically useful in a number of anatomic sites involved by tumor.

Project DescriptionProfessional Personnel Engaged on the Project:

H. Pass	Senior Investigator	SB, NCI
W. Sindelar	Senior Investigator	SB, NCI
W. Friauf	Engineer	BEIB, DES
R. Bonner	Biophysicist	BEIB, DES
P. Smith	Laser Physicist	BEIB, DES
W. Travis	Senior Investigator	LP, NCI
A. Dwyer	Senior Investigator	RB, NCI

Objective

This is a Phase I study designed to assess the toxicity and effectiveness of photodynamic therapy with Photofrin II and laser light in treatment of surface malignancies. Physical parameters of light distribution in tissue are being measured, as well as photosensitizer pharmacology.

Methods Employed

Patients with surface malignancies, cutaneous or mucosal, that are not curable by conventional therapy are eligible for this protocol. Patients receive the Photofrin II photosensitizer by intravenous administration 1.5 - 2.0 mg/kg. Laser light is then delivered in single or multiple fractions to the involved tumor area, using optical fibers for surface illumination, endoscopic treatment, or intraoperative treatment, depending on the patient's clinical problem.

Major Findings

Sixty-one patients have been entered on the protocol. Twenty-six patients have had recurrent cancer in skin. Of these, 20 have had recurrent breast cancer on the chest wall. Five complete responses have been seen in these breast cancer patients but patients recurred 2-9 months after treatment, while 2 other patients had partial responses. This modality is less than optimal for treatment of these recurrent breast cancers because of the limited tissue penetration of the light wavelength currently employed. Other patients successfully treated for skin malignancies include 1 patient with recurrent squamous carcinomas of the head and neck involving the skin, 1 patient with cutaneous lymphoma, and a patient with multiple recurrent Merkel cell carcinoma lesions of the skin of the face. Pigmented melanoma does respond because of heavy pigmentation which attenuates light. One patient with epidemic cutaneous Kaposi's sarcoma has received 2 courses of treatment without response.

Thirteen patients have been treated for endobronchial obstruction. Treatment was considered effective in 11 of 13 patients, with either relief

of bronchial obstruction or maintenance of narrowed but patent airway.

Twenty patients with disseminated intraperitoneal tumors have received the hematoporphyrin derivative prior to laparotomy. Fifteen of the 20 were able to undergo tumor debulking and intraperitoneal photodynamic therapy at progressively increasing doses. Seven of these 15 patients remain free of disease at follow-up times of up to 7 months. There's been no significant toxicity.

Treatment-related morbidity includes sunburn in five patients, full thickness skin necrosis in 2 patients requiring surgical repair or burn treatment, and moderate discomfort in the treatment field requiring medication. In the patients with bronchial lesions, 1 patient died of massive hemoptysis from recurrent tumor 3 months after treatment. The contribution of photodynamic therapy to this complication is uncertain. One patient developed a radiographic infiltrate after treatment which resolved on antibiotics and 1 patient developed a pneumothorax which was successfully treated.

#### Significance to Biomedical Research and the Program of the Institute

Photodynamic therapy represents a potentially curative therapy for selective groups of patients with malignant disease. In particular, patients with a tumor that is accessible to light either by superficial, endoscopic or interstitial illumination may benefit from treatment. Intraoperative treatment is both practical and potentially efficacious. We envision a possible use for this therapy in patients with carcinoma in-situ of the urinary bladder, tumors involving the peritoneal and pleural surfaces, and in selected skin cancers.

#### Proposed Course

We hope to move on to Phase II studies in the following sites: abdomen (peritoneal cavity), bronchus, and esophagus. We are interested in photodynamic therapy in patients with refractory pleural effusions and malignancies. Animal studies are currently in progress as a prelude to such clinical work. Long-range plans also include examination of other photosensitizers which may be activated by light with deeper tissue penetration, and which may have less cutaneous photosensitivity.

#### Publications

1. DeLaney TF, Glatstein E. Photodynamic therapy of cancer, Comprehensive Therapy 1988;14:43-55.
2. DeLaney TF. Photodynamic therapy. In: McGrath I, ed. New directions in cancer treatment, (in press).

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 CM 06380-03 RO
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology of Cellular Injury		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	A. J. Fornace, Jr.	Senior Investigator ROB, NCI
Others:	M. Papathanasiou I. Alamo M. C. Hollander	Visiting Fellow Microbiologist Microbiologist ROB, NCI ROB, NCI ROB, NCI
COOPERATING UNITS (if any) NIA, Baltimore, MD (N. Holbrook); NICHD (D. Nebert); University of Newcastle on the Tyne, U.K. (I. Hickson); University of Utah, Salt Lake City, UT (L. Barrows).		
LAB/BRANCH Radiation Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.8	PROFESSIONAL: 2.4	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>In bacteria and yeast, many of the genes important in the cellular response to DNA damage are induced by such damage. Until recently, evidence for such specific responses to genotoxic stress in mammalian cells was not definitive. In the last year, our group has been in the forefront in demonstrating that certain genes in mammalian cells can be specifically induced by specific types of DNA damage and that SOS-like responses exist in mammalian cells. We developed a hybridization subtraction technique to isolate cDNA clones coding for DNA-damage-inducible (DDI) transcripts, and have isolated cDNA clones for more than 20 different new DDI genes. Most were only induced by DNA damage and not other types of stress such as heat shock. We have sequenced most of these cDNA clones and recently have isolated genomic clones for some of these sequences. One cDNA clone, DDIA18 was found to encode an mRNA only induced by DNA-damaging agents repaired by nucleotide excision (UV-type) repair, and was found to code for a single-stranded-DNA binding protein. cDNA clones for both human and rodent DDIA18 mRNA have been sequenced; the predicted peptide sequence has been very highly conserved which implies that this protein plays an important cellular role. Several other of our DDI genes were found to be coordinately induced by either DNA damage or inhibition of cell growth. There is good evidence in both bacteria and eukaryotes that inhibition of cell growth after DNA damage can have a protective effect; e.g., one of the SOS genes is a growth arrest gene. In collaboration with D. Nebert, we have found that these genes were coordinately overexpressed in a mouse mutant which may provide insight into their regulation. Two of these genes have been sequenced and newly described regulatory regions have been tentatively identified; antibodies to one of the proteins have been developed. In collaboration with I. Hickson and L. Barrows, expression of our DDI transcripts in DNA repair mutants has been investigated. Several examples of both increased and decreased expression transcription in the mutant cells has been observed. The functions of most of our DDI sequences are unknown, but it is probable that the protein products of at least some of these transcripts play a role in the cellular response to DNA damage.</p>		

Project Description

Objective: To isolate DNA-damage-inducible genes in mammalian cells and to study both their function and regulation.

Methods Employed

Standard molecular biology techniques and specialized hybridization subtraction cDNA cloning approach which was developed in this laboratory.

Major Findings

Isolation of more than 20 different cDNA clones that code for DDI transcripts including many that were specifically induced by DNA damaging agents. Many of these genes have been well-conserved (which suggests important functions) since they were expressed and induced in human cells. One cDNA clone, DDIA18 was found to code for a nucleic acid single-stranded binding protein which is DNA-damage inducible. The human equivalent of DDIA18 has been isolated and sequenced; this protein is very highly conserved in humans and rodents. Several examples of abnormal expression were found in DNA repair mutants. Genes for several of our growth arrest DDI clones have been isolated; and two have been sequenced. In the course of our studies, we have also found that the *fos* oncogene is strongly induced by DNA-damaging agents or heat shock in Chinese hamster cells.

Significance to Biomedical Research and the Program of the Institute

DNA damage and its repair play a central role in carcinogenesis and also in the cellular response to many antineoplastic agents. Since our clones code for genes induced by DNA damage, it is likely that their protein products play a role(s) in the response of cells to this type of injury.

Proposed Course

We plan to study the regulation of these genes and the functions of their protein products. We have already isolated and sequenced full-length cDNA and genomic clones for certain DDI genes; studies have been initiated with recombinant expression vectors. With isolation of genomic clones, regulation of these DDI genes can now be studied. In collaboration with N. Holbrook, antibodies have been developed to the protein of one of our genes; this will allow us to isolate protein and study its function.

Publications

1. Fornace AJ Jr, Schach H, Alamo I Jr. Coordinate induction of metallothioneins I and II in rodent cells by UV irradiation, *Molec Cell Biol* 1988;8:4716-20.
2. Fornace AJ Jr, Alamo I Jr, Hollander MC. DNA damage-inducible transcripts in mammalian cells, *Proc Natl Acad Sci USA* 1988;85:8800-4.
3. Hollander MC, Fornace AJ Jr Induction of *fos* RNA by DNA damaging agents, *Cancer Res* 1989;49:1687-92.
4. Bohr VA, Evans MK, Fornace AJ Jr. DNA repair and its pathologic implications, *Laboratory Investigation* 1989 (in press).



5. Fornace AJ Jr, Nebert D, Hollander MC, Papathanasiou M, Fargnoli J, Holbrook N. Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents, *Molec Cell Biol* 1989 (in press).

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 CM 06381-03 RO</div>
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Modeling of Time-Dose Response of Human Tumors and Normal Tissues</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	<b>J. van de Geijn</b>	Radiation Physicist <b>ROB, NCI</b>
Others:	<b>J. Mitchell</b> <b>R. Miller</b> <b>J. Chen</b> <b>E. Glatstein</b>	Radiobiologist <b>ROB, NCI</b> Radiation Physicist <b>ROB, NCI</b> Radiation Physicist <b>ROB, NCI</b> Radiotherapist <b>ROB, NCI</b>
COOPERATING UNITS (if any)		
LAB/BRANCH <b>Radiation Oncology Branch</b>		
SECTION <b>Radiation Physics and Computer Automation Section</b>		
INSTITUTE AND LOCATION <b>NCI, NIH, Bethesda, Maryland 20892</b>		
TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">3.0</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">3.0</div>	OTHER: <div style="text-align: center; font-weight: bold;">0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>The purpose of radiation therapy is tumor control. In view of clonogen proliferation it makes sense to deliver the necessary dose in as short a time as possible. The limiting factor in tumor treatment is normal tissue reaction: normal tissue reactions must not exceed "tolerance" level. Ideally, delivery of treatment is distributed <i>spatially</i> over the smallest volume encompassing the target, and <i>temporally</i> over the shortest time compatible with full, or at least acceptable, recovery of normal tissue functionality. The development and application of high technology particularly in computers and computer-based and assisted imaging has stimulated great progress in tumor localization and treatment planning, and even in the technology of delivery and its quality assurance, i.e., the spatial aspects of the issue. Decision-making as to the amount of dose and its distribution over time (by fractionation or protraction) is still essentially empirical, however. The present project continues the development and exploration of a theoretical description of time-dose response of tumors as well as normal tissues. Its basic concepts have been published in 1988[1]. The current development centers on the implementation of an extension of the conventional Linear-Quadratic model. The extension concerns a unified description of the influence of incomplete repair and comprises an initial description for the influence of proliferation. The present developments cover both high-dose rate fractionated and protracted treatment.</p>		

### Project Description

Objectives: To develop a mathematical formalism describing:

1. The attrition of functioning normal tissue cells.
2. The survival rate, per single dose, of viable stem cells.
3. The inter-fraction and post-treatment course repopulation including an account of the sublethal damage repair, of viable stem cells.
4. The survival rate of clonogenic tumor cells per single-dose.
5. The effective dose for early as well as late reacting normal tissues.
6. The inter-fraction and post-treatment growth pattern of the clonogenic cells, as well as the gross tumor.

### Methods Employed

1. The alpha/beta (2-parameter) model is applied for the single-dose response of stem cells and clonogenic tumor cells.
2. Radiation damage is assumed to consist of a directly lethal and a (repairable) sublethal component.
3. Linear attrition over time is assumed for functioning normal tissue cells as well as non-clonogenic tumor cells.
4. Normal tissue cell loss and replacement is under homeostatic control.
5. Clonogenic tumor cells are assumed proliferate exponentially over time.
6. Stem-cell proliferation is triggered only after some distress signal related to functionality cell levels drop below a certain threshold.
7. Normal tissue tolerance is interpreted as the lower limit of normal tissue functionality: the normal tissue functioning cells dropping below some fraction of their normal count.

### Major Findings

1. A provisional mathematical model has been developed and has proved to be promising.
2. Interactive computer programs have been developed which enable automatic search for acceptable parameters, based on estimates of reasonable ranges of certain key parameters, such as  $D_0$ , cell doubling times, etc.
3. It is possible to stimulate time-dose response patterns for conventional and unconventional fractionation schemes, which are reasonably consistent with published findings in some clinical trials.

4. It is possible to calculate isoeffective doses for late reacting normal tissues, as well as tumors.

#### Significance to Biomedical Research and the Program of the Institute

1. The present model shows promise as a tool toward understanding of time-dose response to conventional or "standard" treatment schedules, as well as some hyper-fractionation schemes and other non-standard schemes.
2. The model promises to become useful to explore, by simulation, other unconventional schemes, and provide reasoned guidance to at least avoid work results especially as regards to late reactions and tumor.

#### Proposed Course

1. Continuation of study of literature data.
2. Design of relevant experiments.
3. Expansion to low dose rate treatment, both single session and fractionated.

#### Publications

1. van de Geijn J. Incorporating the time factor into the linear quadratic model. [Letter to the Editor]. Br J Radiol 1989;62:296-7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 CM 06382-03 R0

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Therapy with Radiolabelled Antibodies: Technical & Dosimetric Aspects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R. Miller	Radiation Physicist	ROB, NCI
Others:	A. Raubitschek	Radiotherapist	ROB, NCI
	J. van de Geijn	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	J. Carrasquillo	Nuclear Medicine Physician	NM, CC

COOPERATING UNITS (if any)

Nuclear Medicine Department, CC; Diagnostic Radiology Department, CC.

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

10.0

PROFESSIONAL

8.5

OTHER

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Administration of radiolabelled antibodies is a relatively new treatment modality for certain forms of cancer. Much of this field is developmental in nature. In particular, the dosimetry of tumor masses, especially at the microscopic level, is at yet unknown. The Radiation Physics and Computer Automation Section is actively assisting in the implementation of clinical protocols. Current research is in two major areas.

Imaging of the organ-specific distribution patterns on a temporal basis is fundamental to the understanding of antibody kinetics and for large volume radiation dosimetry (at the total organ level). The ability to accurately localize biodistribution patterns using nuclear medicine imaging techniques and to accurately register these images with respect to other imaging modalities (CT or MRI) is essential for obtaining quantitative results.

Dosimetry of microscopic tumor masses is approached through the use of computer modeling. The results, where possible, will be validated using quantitative autoradiography.

## Project Description

**Objectives:** To localize the sites of retention of radiolabelled antibodies and to determine the deposition-retention kinetics as well as the clearance pathways. To determine normal organ radiation doses and tumor dose, if possible on a microscopic level for alpha, beta and gamma emitting radionuclides. To determine the optimum combination of imaging modalities for localization and to determine the lower limits of detection of tumor masses with external imaging devices.

## Methods Employed

This project will use small animal models to determine the metabolic pathways of various antibodies and their deposition-retention-excretion kinetics. Phantom studies will be conducted to determine the optimum imaging modalities and their lower limits of detection. These will be confirmed using large animal models. Computer models for determining dose distributions on a microscopic level and for alpha emitting radionuclides will be developed and tested with animal models. Patients under treatment will be imaged, as appropriate, and will be bioassayed using external counting techniques. Biopsies will be taken and used to validate metabolic and dosimetric models for each radiolabelled antibody.

## Major Findings

Studies at other institutions indicate that therapy with radiolabelled antibodies offers little advantage over conventional forms of radiation therapy in the treatment of large tumor masses, due to the inhomogeneous distribution pattern of organ uptake. This results in large dose gradients within the treated site. Antibody therapy shows real promise, however, in the treatment of small tumor masses, especially microscopic disease. The problem with this approach is that the size of these masses makes them difficult to localize using traditional nuclear medicine imaging techniques. It may be possible to image these masses by employing other imaging modalities, either singly or in combination. Also, the dose calculational formalism for distributed radionuclide sources (MIRD), may no longer be valid under these conditions, since the range of the particulate radiations may be greater than the dimensions of the tumor mass and the distribution of radioactivity may be inhomogeneous. A new formalism will need to be developed for alpha emitting radionuclides, as their energy deposition pattern differs significantly from beta-gamma emitters.

## Significance to Biomedical Research and the Program of the Institute

Radiolabelled antibodies are a new, exciting potential treatment modality. They offer the promise of selectively irradiating tumor masses, while delivering minimal radiation doses to normal tissues. This represents the ideal form of radiation therapy. It is possible that, for some forms of cancer, radiolabelled antibody therapy will supplant chemotherapy as the treatment of choice for microscopic disease.

Proposed Course

To be continued. The GE GEMINI SPECT-capable gamma camera originally dedicated to this project is to be replaced by a different unit, due to protracted installation and interfacing problems. A laser system to facilitate patient alignment will be installed in the room where the new camera will be located. Phantom studies will shortly commence, first employing simple geometries, and progressing to humanoid organ phantoms. Image processing techniques will be developed to correlate images from different scanning modalities to aid in diagnosis and treatment planning. Two alternative methods for quantitating whole-body clearance of gamma-emitting radiosotopes will be instituted and compared. The first uses a dedicated microcomputer with both multichannel analysis and multichannel scaling capabilities, while the latter is a much simpler, less expensive system utilizing a portable, data-logging radiation detector.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 CM 06383-03 R0</b>																				
PERIOD COVERED <b>October 1, 1988 to September 30, 1989</b>																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Development of an Improved Treatment Chair for Radiation Therapy</b>																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%; vertical-align: top;">PI:</td> <td style="width: 35%; vertical-align: top;">R. Miller</td> <td style="width: 35%; vertical-align: top;">Radiation Physicist</td> <td style="width: 15%; vertical-align: top;">ROB, NCI</td> </tr> <tr> <td style="vertical-align: top;">Others:</td> <td style="vertical-align: top;">A. Raubitschek</td> <td style="vertical-align: top;">Radiotherapist</td> <td style="vertical-align: top;">ROB, NCI</td> </tr> <tr> <td></td> <td style="vertical-align: top;">F. Harrington</td> <td style="vertical-align: top;">Biomed. Engineering Tech.</td> <td style="vertical-align: top;">ROB, NCI</td> </tr> <tr> <td></td> <td style="vertical-align: top;">J. van de Geijn</td> <td style="vertical-align: top;">Radiation Physicist</td> <td style="vertical-align: top;">ROB, NCI</td> </tr> <tr> <td></td> <td style="vertical-align: top;">J. Ovadia</td> <td style="vertical-align: top;">Visiting Scientist</td> <td style="vertical-align: top;">ROB, NCI</td> </tr> </table>			PI:	R. Miller	Radiation Physicist	ROB, NCI	Others:	A. Raubitschek	Radiotherapist	ROB, NCI		F. Harrington	Biomed. Engineering Tech.	ROB, NCI		J. van de Geijn	Radiation Physicist	ROB, NCI		J. Ovadia	Visiting Scientist	ROB, NCI
PI:	R. Miller	Radiation Physicist	ROB, NCI																			
Others:	A. Raubitschek	Radiotherapist	ROB, NCI																			
	F. Harrington	Biomed. Engineering Tech.	ROB, NCI																			
	J. van de Geijn	Radiation Physicist	ROB, NCI																			
	J. Ovadia	Visiting Scientist	ROB, NCI																			
COOPERATING UNITS (if any)																						
LAB/BRANCH <b>Radiation Oncology Branch</b>																						
SECTION <b>Radiation Physics and Computer Automation Section</b>																						
INSTITUTE AND LOCATION <b>NCI, NIH, Bethesda, MD 20892</b>																						
TOTAL MAN-YEARS <b>3.5</b>	PROFESSIONAL: <b>2.0</b>	OTHER <b>1.5</b>																				
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																						
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             This project is intended to design a treatment chair which overcomes the inherent design limitations of commercially available chairs. This chair will function independently of the treatment couch so as to permit opposed field treatment in any orientation in an extended isocentric fashion (the center of rotation will be at a distance greater than the standard isocenter of the accelerator). It will be capable of accurate, reproducible rotation and translation in the lateral, longitudinal and vertical planes. If possible, the chair will function with a standard radiotherapy simulator to permit proper localization, immobilization and treatment planning.           </p> <p>             The chair is being designed on the "tool platform" principle. That is, the chair will function as a platform, allowing the attachment of various additional devices which can be placed in such a manner that they permit proper immobilization of the patient without unduly restricting treatment delivery.           </p>																						



## Project Description

**Objectives:** To develop an independent treatment chair to permit multiple-field radiation therapy at either standard or extended SSD.

## Methods Employed

The Radiation Therapy Machine Shop fabricates any chair components and accessories that are needed. Selected patients are placed in the chair for simulation and for their course of therapy. Any problems associated with immobilization and repositioning are analyzed on a daily basis and the necessary modifications are made.

## Major Findings

The initial version of the treatment chair permitted opposed-field treatments and could be used with the simulator as well as with any treatment unit. Treatment of some forms of cancer with the patient seated is advantageous. The original design of the chair was excessively limited by the stipulation that it operate with the simulator. The hydraulic vertical motion was imprecise and the positioning of the patient by hand was operationally difficult. The center of rotation of the chair was also at an undesirable location, which precluded its use in an isocentric manner. A new elevating mechanism has been designed and implemented. This consists of an electrically-driven, precision scissors which provide a great degree of stability and a more precise control of vertical position. Patient positioning has been improved by using a back rest and a stylastic seat cushion. A new back rest "Tennis Racket" is being built to allow for the marking of posterior set-up and alignment points on the patient. An "extended isocentric" mounting is being explored for the chair. This will provide for complete clearance of all obstacles, which in the past have limited the rotational freedom of the chair. A distance of up to 120 cm. can be accommodated with the current simulator.

## Significance to Biomedical Research and the Program of the Institute

Treatment of the mediastinum with the patient seated can minimize the amount of lung in the irradiated field, minimizing complications. A combined Waldeyer's/mantle field treatment is possible in this position. Also, low dose rate mantle fields can be used by placing the chair at an extended SSD.

## Proposed Course

Currently, the chair has undergone a major modification which incorporates a base-mounted turntable to provide isocentric positioning of the patient. This greatly simplifies the initial set-up and treatment. The vertical stability has also been greatly improved. These modifications still permit the chair to be used with our current simulator. A new elevating mechanism

will be developed to give precise control over patient positioning in the vertical direction. Also, the possibility of providing motor driven controls will be explored and additional attachments for positioning and immobilizing the patient will be developed.

Publications

None.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06384-02 RO

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Regulation and cDNA Cloning of DNA Polymerase  $\beta$  in Chinese Hamster Cells

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A. J. Fornace, Jr.	Senior Investigator	ROB, NCI
Others:	I. Alamo	Microbiologist	ROB, NCI
	M. C. Hollander	Microbiologist	ROB, NCI

## COOPERATING UNITS (if any)

NCI (S. Wilson).

## LAB/BRANCH

Radiation Oncology Branch

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.3

## PROFESSIONAL

0.2

## OTHER

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

$\beta$ -polymerase is one of the few mammalian DNA repair genes which have been isolated. This enzyme is responsible for the repair polymerase step ("gap filling" step) of DNA repair. In the case of short patch repair after damage by base damaging agents such as alkylating agents,  $\beta$ -polymerase is the only polymerase utilized. The genes of repair polymerases in E. coli and yeast have been found to be DNA-damage inducible. In collaboration with S. Wilson who has cloned both human and rat  $\beta$ -polymerase cDNA, we have found that  $\beta$ -polymerase RNA is rapidly induced in Chinese hamster ovary (CHO) cells after exposure of the cells to alkylating agents or hydrogen peroxide. Induction did not occur after UV radiation, heat shock, perturbation of cell cycle, or exposure to other DNA damaging agents which did not induce high levels of adducts to single bases in DNA. This is the first demonstration of the induction of a DNA repair gene in higher eukaryotic cells specifically by DNA damage. In order to further elucidate the regulation of  $\beta$ -polymerase in CHO cells, we have isolated the  $\beta$ -polymerase cDNA clone from a CHO cDNA library. Sequence analysis demonstrated that  $\beta$ -polymerase has been highly conserved in the Chinese hamster, rat, and human species. In collaboration with S. Wilson, we have found evidence that a DNA-damage-inducible trans-acting protein binds to the  $\beta$ -polymerase promoter, and can lead to increased transcription of this gene.

### Project Description

Objective: To study the regulation of  $\beta$ -polymerase gene in Chinese hamster cells.

### Methods Employed

Standard molecular biology approaches.

### Major Findings

The Chinese hamster  $\beta$ -polymerase gene is the first mammalian DNA repair which has been found to be specifically induced by DNA-damaging agents. We have cloned and sequenced a Chinese hamster cDNA clone for  $\beta$ -polymerase. Evidence has been found for a DNA-damage responsive element in the promoter of the  $\beta$ -polymerase gene.

### Significance to Biomedical Research and the Program of the Institute

$\beta$ -polymerase plays an important role in the repair of damage by DNA damaging agents such as alkylating agents. Characterization of this gene and its regulation will probably provide insight into the response of mammalian cells to agents with relevance to both cancer therapy and carcinogenesis.

### Proposed Course

See summary of work.

### Publications

1. Zmudka BZ, Fornace A, Collins J, Wilson SH. Characterization of DNA polymerase  $\beta$  mRNA: cell-cycle and growth response in cultured human cells, Nucleic Acids Res 1988;16:9587-96.
2. Fornace AJ Jr, Zmudka BZ, Hollander MC, Wilson, SH. Induction of mammalian  $\beta$ -polymerase mRNA by DNA damaging agents in Chinese hamster ovary cells, Molec Cell Biol 1989;9:851-3.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 CM 06385-02 RO
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Increased Expression of Stress-induced Genes in Chemoresistant Tumor Cells</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	A. J. Fornace, Jr.	Senior Investigator ROB, NCI
Others:	M. C. Hollander M. Papathanasiou	Microbiologist Visiting Fellow ROB, NCI ROB, NCI
COOPERATING UNITS (if any) Fox Chase Cancer Center, Philadelphia, PA (T. Hamilton); Smith, Kline, & French Laboratories, Philadelphia, PA (K. B. Tan); University of Berkeley, CA (R. Goth-Goldstein).		
LAB/BRANCH Radiation Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.4	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)  <p>A major problem in cancer chemotherapy is the emergence of drug-resistant cells. Chemotherapy-resistance is probably due to multiple mechanisms including altered uptake/excretion of drug, increased inactivation of drug, and altered host response such as increased DNA repair. As outlined in project number Z01 CM 06380-03 RO, we have isolated a variety of mammalian cDNA clones which code for transcripts specifically induced by DNA damage. We have initiated studies with a variety of human tumor cell lines which have been selected for resistance to cis-Pt diamminedichloride (DDP), nitrogen mustard (HN2), or other alkylating agents. Our initial studies have involved determining the level of different stress-induced transcripts in these chemoresistant tumor cell lines compared to their parent cell lines with normal sensitivity. Several examples of over-expression of certain of our DDI transcripts in DDP and alkylating agent resistant cells have been found. For example, in the case of DDIA33 RNA, this transcript was constitutively elevated in both DDP, melphalan, and HN2 resistant human tumor cell lines and also in a MNNG resistant Chinese hamster cell line. DDIA18 mRNA was found to be substantially elevated in certain chemoresistant human ovarian cell lines. These studies raise the important possibility that over-expression of certain DNA-damage-inducible genes may play a role in chemotherapy resistance.</p>		

### Project Description

**Objective:** To determine expression of DNA-damage-inducible transcripts in chemotherapy resistant tumor cells, and ultimately their role in chemotherapy resistance.

### Methods Employed

Standard molecular biology techniques.

### Major Findings

Several of our *DDI* transcripts have been found to be elevated in chemoresistant tumor cell lines. It was interesting that *DDIA18* mRNA was clearly elevated in several DDP-resistant ovarian cell lines. We have found that the level of this mRNA was unaffected by cell growth or cell cycle, and that the level of this mRNA was fairly constant in a variety of different cell lines. As described in project number Z01 CM 06380-03 RO, *DDIA18* mRNA was only induced by DNA-damaging agents whose damage is repaired by nucleotide excision repair. The important lesions in DNA induced by DDP (interstrand crosslinks and intrastrand crosslinks) are removed by nucleotide excision repair.

### Significance to Biomedical Research and the Program of the Institute

Identification of genes and regulatory pathways involved in certain forms of chemotherapy resistance may lead to better understanding of this serious clinical problem, and may ultimately provide insights into overcoming this problem.

### Proposed Course

The short-term approach will be to determine *DDI* transcript levels in a variety of chemotherapy resistant cells and attempt to correlate this with cellular resistance. The long-term course involves a thorough characterization of these genes and their regulation.

### Publications

None.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06386-02 RO

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Radioimmunotherapy of Peritoneal Cancer with I-131 Labeled B 72.3

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A. Raubitschek	Senior Investigator	ROB, NCI
Others:	J. Carrasquillo	Head, Antibodies Project	NM, CC
	R. Neumann	Chief	NM, CC
	J. Reynolds	Senior Investigator	NM, CC
	J. Schlom	Chief	LTIB, NCI
	D. Colcher	Senior Investigator	LTIB, NCI

## COOPERATING UNITS (if any)

## LAB/BRANCH

Radiation Oncology Branch

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

.5

## PROFESSIONAL

## OTHER

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

In cooperation with the Department of Nuclear Medicine, and the Laboratory of Tumor Immunology and Biology, we have initiated clinical trials for the treatment of peritoneal carcinomatosis. A classical phase one study has begun using escalating doses of I-131 labeled antibody administered intra-peritoneally.

Since last year's report, we have continued to follow the initial eight patients and have treated an additional two patients. Of the eight patients followed for greater than six months, all but one has clinical evidence of progressive disease. The one patient who is without evidence of disease has not yet been explored surgically; however, he is free of intraabdominal disease on both CAT scans and by serology.

There continues to be minimal toxicity of the treatment with the major dose limiting structure being the bone marrow. Patients who have been extensively pretreated with chemotherapy have exhibited grade three thrombocytopenia. The bone marrow will probably limit the administered dose to 150 millicuries.

We have recently treated a patient who developed bacterial peritonitis following her imaging dose of radiolabeled antibody. This was successfully treated with antibiotics without significant sequelae. We have not yet administered the therapeutic dose.

During this reporting period, we have written addenda to the current protocol allowing for the use of chimeric B72.3 and newer monoclonals with higher binding affinities. In the future, we hope to treat with these newer monoclonals coupled to both radiolabeled iodine and radioactive heavy metals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06387-02 RO

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Superoxide Dismutase Mimics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	A. Samuni	Visiting Scientist	ROB, NCI
	C. Black	Associate	ROB, NCI
	C. Krishna	Associate	ROB, NCI
	E. Bernstein	Associate	ROB, NCI
	J. B. Mitchell	Senior Investigator	ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH Radiation Oncology Branch

SECTION Experimental Phototherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL

OTHER

5

5

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There has been recent interest in the administration of superoxide dismutase (SOD) for ablation of oxygen mediated tissue damage. It is known that oxygen radicals such as superoxide and hydroxyl radical are important radiolysis products in an oxygenated aqueous environment. To date, there is no means of easily studying the effects of augmenting SOD cells. We have found that certain nitroxides, particularly oxazolidine containing nitroxides, may act as low molecular weight, cell permeable, superoxide dismutase mimics. We have demonstrated pH dependence of the oxazolidine nitroxide (OXANO) SOD mimic in both a Xanthine oxidase and cesium source superoxide generating systems. Similarly, the rates of SOD like activity of the oxan system has been determined. The scope of the chemistry is being investigated by synthesizing analogues. Likewise, the biochemistry of the reaction is under investigation. *In vitro* studies have revealed that these nitroxides are: 1) non-toxic; 2) provide protection against hydrogen peroxide toxicity; and 3) penetrate the cell membrane. *In vivo* toxicity are underway.



Project Description

**Objectives:** To create simple, low-molecular weight, metal-independent chemical systems which mimic the chemical behavior of superoxide dismutase. To study the role of such mimics as they might relate to radiation and chemotherapeutic drug modulating and modifying agents. To investigate reactions of the spin-trapping agent DMPO once it has reacted with superoxide anion radical and show that interpretation of such chemistry is germane to the interpretation of results relating to radiation and drug anticancer treatment.

Methods Employed

Electron spin resonance spectroscopy allows the study of free radical chemistry and biology. The study of short lived oxy-radicals (spin trapping) or the rate of interaction of superoxide with oxazolidine nitroxides (stable spin labels) is well suited to the use of electron spin resonance. Organic synthesis of different oxazolidine nitroxides will follow straight forward procedures. UV, NMR, IR, and Mass spectroscopy will be used to characterize the chemical nature of the compounds. Cell culture techniques will be used to evaluate drug and radiation modulation. Immediate use of polymorphonuclear white blood cells to investigate the oxygen burst phenomenon and the interpretation of DMPO reactions, as well the use of superoxide dismutase mimics in changing the effects of the oxygen burst. Murine systems will be used to investigate the pharmacology, biodistribution, and metalism of the different nitroxides.

Major Findings

DMPO-OH is degraded rapidly to a non-esr signal compound by DMPO. Such findings indicate that caution must be used in the interpretation of data relating to hydroxyl radical production when using the commonly employed spin trapping agents. 2,3,3-trimethyl-2-ethyl ox(3)azo(1)lidine nitroxide is the first example of a low-molecular weight, non-metal superoxide dismutase mimic. The reaction is dependent on both the superoxide reduction of the nitroxide and subsequent superoxide oxidation of the reduction product, hydroxyl amine. The reaction is pH dependent (optimal conditions are from 7 to 7.5) and the rate constants are in the order of 10<sup>5</sup> (lower than superoxide dismutase, but much higher concentrations are attainable; therefore, effective catalysis is higher for the superoxide dismutase mimic). In vitro studies reveal that the nitroxides are non-toxic and protect against hydrogen peroxide cytotoxicity.

Significance to Biomedical Research and the Program of the Institute

A better and more complete understanding of the limitations of the common tools used to study ionizing radiation and chemotherapeutic drug effects on biological systems. The superoxide dismutase mimic may have applications in the area of coronary reperfusion, arthritis treatment, inflammation resolution, and decreasing harmful effects of the anthracyclines and bleomycin antineoplastic agents (respective cardiac and lung toxicities), as as well as changing the dose response to radiation-induced damage.

### Proposed Course

To explore the breath of the chemical and biochemical reactions of the first oxazolidine nitroxide. To synthesize analogues which have different characteristics to explore the cellular effects of having superoxide dismutase activity in different sites (membrane, cytoplasm, nucleus, mitochondria) and to investigate the dose modifying effects of such superoxide dismutase mimic on ionizing radiation and chemotherapy drugs.

### Publications

1. Samuni A, Krishna CM, Riesz P, Finkelstein E, Russo A. A novel metal-free low molecular weight superoxide dismutase mimic, J Biol Chem 1988;263(suppl 34): 17921-4.
2. Samuni A, Black CD, Krishna CM, Malech HL, Bernstein EF, Russo A. Hydroxyl radical production by stimulated neutrophils reappraised. J Biol Chem 1988;263(suppl 27):13797-801.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06388-02 R0

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Treatment of Superficial Carcinoma of the Bladder with Photoradiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	E. Glatstein	Branch Chief	ROB, NCI
	A. Russo	Senior Investigator	ROB, NCI
	L. Dachowski	Nursing Clinician	ROB, NCI
	G. Thomas	Microbiologist	ROB, NCI

## COOPERATING UNITS (if any)

## LAB/BRANCH

Radiation Oncology Branch

## SECTION

Radiation Therapy Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

1.5

## PROFESSIONAL

1.5

## OTHER

0.5

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Bladder cancer is subdivided into four groups: 1) Superficial high-grade disease (T1S); 2) superficial disease (T1); 3) superficially invasive into the muscle (T2); and 4) tumor extending into deep muscle and/or perivesical fat (T3A/T3B). Standard therapy for superficial disease confined to the mucosa or submucosa consists of transurethral resection and intravesical chemotherapy (thiotepa, mitomycin C, BCG). Recurrence rates may range from 30%-85% depending upon the grade of tumor and multiplicity of lesions. The concept of a full field defect in patients with carcinoma in-situ in association with a solitary papillary tumor is supported by the high incidence of invasive disease developing within two years following resection alone. Five-year survival rates for patients developing invasive disease (T2/T3A) range from 31%-52%. Early control of superficial disease offers a potential advantage towards reduction of the overall death rate in bladder malignancy. Carcinoma in-situ refractory to intravesical chemotherapy is a particularly troublesome clinical entity, as patients are at high risk for the development of invasive disease and may require removal of the urinary bladder (cystectomy). Recent work with hematoporphyrin derivative (HpD) sensitized photodynamic therapy of the bladder mucosa suggests high cytotoxic effect, but low systemic toxicity. This modality may permit treatment of superficial carcinoma of the bladder as well as carcinoma in-situ which may permit bladder preservation with cure of tumor.

Project DescriptionProfessional Personnel Engaged on the Project:

W. M. Linehan	Senior Investigator	SB, NCI
M. Walther	Senior Investigator	SB, NCI
W. Friauf	Engineer	BEIB, DES
R. Bonner	Biophysicist	BEIB, DES
P. Smith	Laser Physicist	BEIB, DES

Objective

This is a Phase I trial designed to determine the feasibility of treating patients with superficial bladder carcinoma with a combination of hematoporphyrin derivative ( $H_pD$ ) and laser light, and to judge tumor response.

Methods Employed

Eligible patients will receive hematoporphyrin derivative by intravenous injection. They will subsequently undergo a cystoscopy at which time light will be delivered to the involved portion of the bladder according to the clinical judgment of the treating physicians using an optical fiber coupled to an argon pumped dye laser which has been introduced into the bladder through the cystoscope. Following treatment, both cystoscopy and urine cytology will be regularly done to assess response to treatment. If partial responses are observed without serious side effects, repeat treatment will be performed. Patients who develop invasive bladder carcinoma will be taken off protocol and referred for appropriate treatment.

Major Findings

To date, no patients have been entered onto this protocol, although patients are expected to be treated in the near future. Light diffusing fibers which can be introduced into the cystoscope have been procured and calibrated for patient treatment. A specially designed cystoscope holder has been manufactured and described in a recent publication, which can be used for centering the optical fiber during photodynamic therapy at the time of cystoscopy.

Significance to Biomedical Research and the Program of the Institute

Photodynamic therapy represents a potentially useful mode of curative therapy for selected patients with superficial carcinoma of the bladder. If this is achievable without requiring that the patients have their urinary bladder removed, this will represent a major advance in treatment of superficial carcinoma of the bladder.

Proposed Course

We propose to begin accruing patients for photodynamic therapy in the next few months. Twelve patients are currently approved for study on the protocol.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06389-02 RO

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Influence of Lung Density in Mantle Technique Chest Irradiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	A. Raubitschek	Radiotherapist	ROB, NCI
	R. Miller	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	K. Yeakel-Orr	Dosimetrist	ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3

PROFESSIONAL:

1

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Radiation therapy aims at complete destruction of the proliferative capability of all clonogenic tumor cells, while not exceeding the tolerance for radiation damage to any of the tissues and organs. The biological effects depend on the amount and distribution of absorbed dose. Clinical dosimetry depends almost entirely on an underlying theoretical model. In chest irradiation, the field size, and shape dependent dose distribution is further determined by the size and shape of the patient, as well as density distribution, and inside the irradiated volume. The need for accurate accounting for the latter is still not unequivocally recognized. The present project is designed to shed new light on this problem.

The dosimetry of a large number of patients is being investigated using computer generated dosimetry, as well as relevant measurements.

### Project Description

Objectives: To determine the influence of the presence of inhomogeneous tissues in irregularly shaped fields in the treatment of Hodgkin's disease in the chest.

### Methods Employed

1. Generation of dose distributions in multiple sections as many patients as deemed necessary, with and without correction for lung density; CT-backed documentation is available on over 100 patients.
2. Generation of Dose-Volume histograms for both conditions.
3. Performance of adequate supporting measurements in suitable phantom conditions.
4. Two or more different density correction methods will be compared.

### Major Findings

Although many computerized treatment planning systems are commercially or otherwise available, and accurate anatomical data can be obtained from CT, correction for lung density in calculated dose distributions is not common practice. Yet, in order to properly evaluate clinical results, it would seem that arguments either way would be supported by systematic investigative results.

### Significance to Biomedical Research and the Program of the Institute

Evaluation of results of radiation treatment regimens require accurate dosimetry data on individual patients. Depending on the outcome of the proposed work, guidelines might emerge to facilitate more meaningful use of data from inter-institutional studies.

### Proposed Course

1. Available patient material will be used to simulate dose distributions for an adequate spectrum of cases, using different correction methods in common usage, in addition to one locally developed method.
2. Suitable phantom measurements will be performed.

### Publications

In preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 CM 06390-01 R0

PERIOD COVERED  
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Bifunctional Chelates for Gallium (III)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. McMurry

Staff Fellow

ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH  
Radiation Oncology Branch

SECTION  
Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION  
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS  
0.4

PROFESSIONAL  
0.4

OTHER  
0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have initiated a project with the intent of developing bifunctional chelates to specifically sequester trivalent radionuclides such as Gallium (III). Bifunctional chelates provide a means for conjugating radionuclides to monoclonal antibody, resulting in a potentially site-specific radiopharmaceutical. To this end, we have chosen to synthesize new bifunctional chelates which incorporate functionalized catechol (1,2-dihydroxybenzene) binding subunits. Chelates of this type form highly stable 3:1 complexes with trivalent metals at physiological pH, yet typically have little affinity for divalent metals, thus making the tris (catecholates) inherently more selective complexing agents than the more familiar polyaminocarboxylates (e.g., EDTA).

We have recently completed the synthesis of a new macrocyclic tris (catecholate) chelate which incorporates a side arm for attachment to monoclonal antibody. Preliminary thin layer chromatography evidence indicates the chelate efficiently extracts Ga-67 from Ga-67 (citrate) at pH 6.6.

Current experiments involve the synthesis of C-14 labeled chelate and coupling of the chelate to monoclonal antibody through either an isothiurea linkage or via reductive amination of oxidized carbohydrate residues on the antibody.



Project Description

## Professional Personnel Engaged on the Project:

S. Mirzadeh  
O. Gansow

Cancer Expert  
Senior Investigator

ROB, NCI  
ROB, NCI

**Objectives:** We plan to evaluate the utility of the new bifunctional tris (catecholate) chelate for labeling of monoclonal antibody with Gallium (III). This broad objective includes the evaluation of the stability of the metal complex, conjugation with protein, and eventual in vivo studies.

The thermodynamic stability of the Gallium complex will be determined by classical techniques and the metal exchange properties investigated. These data will help us predict whether or not the integrity of the metal complex will be compromised in vivo. Conditions for optimal conjugation of the chelate to antibody will be investigated as will the techniques for labeling with several Gallium isotopes (Ga-66, 67, 68).

Since the Ga-68, 66 radionuclides could be useful for diagnosis by PET and for therapy, respectively, parallel in vivo studies on animal tumor models will be performed with Ga-67, a readily available gamma emitter.

One specific goal of the project is to make the Gallium isotopes useful for PET imaging and consequent accurate dosimetry when delivered to tumor by monoclonal antibody. Thus, when large doses of Ga-66 are subsequently used for tumor therapy, an accurate correlation between dose and therapeutic efficacy may be made.

These new chelating agents are also potentially useful for linkage of the 10.6 hour lead-212 isotope which could deliver alpha-particles to tumors when linked to monoclonal antibody.

We anticipate that these new methodologies will be most useful for the treatment of AIDS-related lymphoma and other blood borne malignancies.

Methods Employed

Standard organic and inorganic synthetic techniques are required for the preparations of the chelate. Evaluation of the labeling efficiency will be achieved using radiochemical tracers (C-14, Ga-67) and UV-VIS spectroscopy.

Major Findings

We have synthesized the first macrocyclic tris (catecholate) chelate with appropriate functionality necessary to attach the chelate to monoclonal

antibody. The efficacy of this ligand is demonstrated by its ability to remove Ga-67 from the citrate ligand at pH 6.6.

#### Significance to Biomedical Research and the Program of the Institute

Several Gallium isotopes have desirable properties for applications in nuclear medicine. In particular, Ga-67 78.3 hr, (EC 100%, 93(38%), 185(24%) KeV) and Ga-68 (68 min., B<sup>+</sup>, 90% (1.89 MeV,100%) are suitable for gamma imaging and PET scanning, respectively, while the energetic positron emission of Ga-66 (9.45 hr, B<sup>+</sup>,56%(4.2 MeV,51.2%), EC 44%) combined with its half-life of 9.5 hours make it an attractive candidate for radioimmunotherapy. While simple inorganic complexes (e.g., Ga-67 (citrate)) of Ga-67 and Ga-68 are used clinically, it is anticipated that conjugation with monoclonal antibody will greatly enhance the utility of Gallium isotopes. By developing a selective and stable bifunctional chelate for attachment of Gallium to monoclonal antibody, we hope to contribute to the development of site-specific radiopharmaceuticals, in particular, for the treatment of AIDS-related lymphoma.

#### Publication

McMurry TJ, Raymond KN, Smith PH. Molecular recognition and metal ion template synthesis. Science 1989;244:938-43.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 03800-19 SURG

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Surgical Consultants &amp; Collaborative Research Involving Surgical Services at NIH

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: S.A. Rosenberg

Chief of Surgery, NCI

SURG, NCI

Others: Entire Staff

Surgery Branch

SURG, NCI

## COOPERATING UNITS (if any)

GD Aurbach (NIAMDD), JL Doppman (CC), E Glatstein (NCI), J Robbins (NIAMDD),  
L Liotta (NCI), RC Young (NCI), P Pizzo (NCI), J Gardner (NIAMDD)

## LAB/BRANCH

Surgery Branch

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

5.0

## PROFESSIONAL:

5.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided)

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty surgical problems except for the specialties of cardiac and orthopedic surgery. The Surgery Branch answers all emergency as well as elective surgical consultations and provides 24 hour coverage for surgical emergencies that may arise in the Clinical Center Hospital.

Secondly, the Surgery Branch collaborates in the procurement of tissues for studies required by other investigative units. The degree of involvement of the Surgery Branch in the planning and execution of these studies is variable. The Surgery Branch often plays an instrumental role in the design of these studies while in other collaborations, the Surgical Service merely provides tissues.

Approximately 40% of the clinical surgical effort of the Surgery Branch is devoted to these consultative and collaborative studies.

A complete listing of surgical procedures performed by the Surgery Branch is presented in Table I.

Over 1000 consultations were received last year from other NCI Branches as well as other NIH Institutes.

# SURGICAL SERVICES DEPARTMENT

## ANNUAL STATISTICS

APRIL 1988 - MARCH 1989

TOTAL PROCEDURES	HOURS	INSTITUTES/OTHERS	TOTAL PROCEDURES
<u>431-1/2</u>	<u>1189.00</u>	Ward (NCI)	<u>150</u> Emergencies
<u>941</u>	<u>2094.25</u>	Consult (NCI)	<u>170</u> Add-ons
<u>74</u>	<u>138.00</u>	Med. Br. (NCI)	<u>597</u> Cancellations
<u>1446-1/2</u>	<u>3421.25</u>	TOTAL (NCI)	<u>336</u> OPD's
			<u>29</u> 2WCSR
<u>1446-1/2</u>	<u>3421.25</u>	NCI	<u>1</u> ICU-2J
<u>323-1/2</u>	<u>1474.25</u>	NHLBI	<u>7</u> MICU-10D
<u>179-1/2</u>	<u>952.50</u>	NINCDS	<u>32</u> Other Radiation
<u>40</u>	<u>38.25</u>	Med. Neuro	
<u>77</u>	<u>177.00</u>	NEI	
<u>78-1/2</u>	<u>164.75</u>	ENT	
		ROB	<u>2274</u> Total Cases
<u>37</u>	<u>139.75</u>	NIDR	<u>6540.25</u> Total Hours
<u>11</u>	<u>40.00</u>	Orthopedics	
<u>68-1/2</u>	<u>117.00</u>	NICHD	
<u>12-1/2</u>	<u>15.50</u>	Other (Cattau, Trout, Latimer, Brauer, Kozloff)	

## MONTHLY SUMMARY

January	<u>181</u>	Total Procedures	July	<u>188</u>	Total Procedures
	<u>524.00</u>	Total Hours		<u>562.00</u>	Total Hours
February	<u>157</u>	Total Procedures	August	<u>215</u>	Total Procedures
	<u>453.50</u>	Total Hours		<u>591.00</u>	Total Hours
March	<u>195</u>	Total Procedures	September	<u>204</u>	Total Procedures
	<u>575.50</u>	Total Hours		<u>548.50</u>	Total Hours
April	<u>193</u>	Total Procedures	October	<u>186</u>	Total Procedures
	<u>500.50</u>	Total Hours		<u>599.25</u>	Total Hours
May	<u>191</u>	Total Procedures	November	<u>192</u>	Total Procedures
	<u>513.00</u>	Total Hours		<u>544.75</u>	Total Hours
June	<u>192</u>	Total Procedures	December	<u>180</u>	Total Procedures
	<u>608.25</u>	Total Hours		<u>520.00</u>	Total Hours

## PUBLICATIONS

Z01 CM 03800-19 SURG

1. Norton JA, Cromack DT, Shawker TH, Doppman JL, Comi R, Gorden P, Maton PN, Gardner JD, Jensen RT. Intraoperative ultrasonographic localization of islet cell tumors, *Ann Surg* 1988;207:160-8.
2. Avis FP, Ellenberg S, Friedman MA. Surgical oncology research. A dis-appointing status report, *Ann Surg* 1988;207:262-6.
3. Rizzoni WE, Miller K, Rick M, Lotze MT. Heparin-induced thrombocytopenia and thromboembolism in the postoperative period, *Surg* 1988;103:470-6.
4. Fraker DL, Norton JA. Localization and resection of insulinomas and gastrinomas, *JAMA* 1988;295:3601-5.
5. Darling GE, Marx SJ, Spiegel AM, Aurbach GD, Norton JA. Prospective analysis of intraoperative and postoperative urinary cyclic adenosine 3' 5'-monophosphate levels to predict outcome of patients undergoing reopera-tions for primary hyperparathyroidism, *Surg* 1988;104:1128-36.
6. Fraker DL, Norton JA, Saeed AA, Maton PN, Jensen RT. A prospective study of perioperative and postoperative control of acid hypersecretion in pa-tients with Zollinger-Ellison syndrome undergoing gastrinoma resection, *Surg* 1988;104:1054-63.
7. Edington HD, Evans S, Sindelar WF. Reconstruction of a functional hemi-diaphragm with use of omentum and latissimus dorsi flaps, *Surg* 1989;105:442-5.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03801-19 SURG

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Clinical Studies in Cancer Surgery

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: S.A. Rosenberg

Chief of Surgery, NCI

SURG, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

5.0

PROFESSIONAL:

5.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Surgery Branch has a variety of studies investigating innovative therapies for patients with malignant diseases. The major emphasis of these studies is in the treatment of soft tissue sarcomas, osteogenic sarcomas, colorectal cancer, gastric cancer, renal cell cancer and melanoma. The major emphasis in Surgery Branch cancer therapy is in adjuvant therapy with emphasis on the use of combined treatment modalities in addition to surgery.

1. Rong GH, Sindelar WF. Aberrant peripancreatic arterial anatomy. Considerations in performing pancreatectomy for malignant neoplasms, *Am Surg* 1987;53:726-9.
2. Linehan WM. Editorial. Renal cell carcinoma, *J Urol* 1988;139:340-1.
3. Sindelar WF, Hoekstra HJ, Kinsella TJ. Surgical approaches and techniques in intraoperative radiotherapy for intra-abdominal, retro-peritoneal, and pelvic neoplasms, *Surg* 1988;103:247-56.
4. Rosenberg SA. Immunotherapy of cancer using interleukin-2: Current status and future prospects, *Immunol Today* 1988;9:58-62.
5. Rosenberg SA. Development of new immunologic approaches to cancer therapy, *Blood Purification* 1988;6:69-76.
6. Hoekstra HC, Restepo C, Kinsella TJ, Sindelar WF. Histopathological effects of intraoperative radiotherapy on pancreas and adjacent tissues: A postmortem analysis, *J Surg Oncol* 1988;37:104-8.
7. Kern KA, Norton JA. Reviews: Cancer Cachexia, *J Parenteral and Enteral Nutrition* 1988;12:286-98.
8. Pogrebniak HW, Stovroff M, Roth JA, Pass HI. Resection of pulmonary metastases from malignant melanoma: Results of a 16-year experience, *Ann Thorac Surg* 1988;46:20-3.
9. Chang AE, Kinsella T, Glatstein E, Baker AR, Sindelar WF, Lotze MT, Danforth DN Jr, Sugarbaker PH, Lack EE, Steinberg SM, White DE, Rosenberg SA. Adjuvant chemotherapy for patients with high-grade soft-tissue sarcomas of the extremity, *J Clin Oncol* 1988;6:1491-1500.
10. Lefor AT, Merino MM, Steinberg SM, Dwyer A, Roth JA, Flanagan M, Pass HI. Computerized tomographic prediction of extraluminal spread and prognostic implications of lesion width in esophageal carcinoma, *Cancer* 1988;62:1287-92.
11. Hoekstra HJ, Sindelar WF, Kinsella TJ. Surgery with intraoperative radiotherapy for sarcomas of the pelvic girdle: A pilot experience, *Int J Rad Oncol Biol Phys* 1988;15:1013-16.
12. Sindelar WF. Clinical experience with regional pancreatectomy for adenocarcinoma of the pancreas, *Arch Surg* 1989;124:127-32.
13. Cromack DT, Maher MM, Hoekstra H, Kinsella TJ, Sindelar WF. Are complications in intraoperative radiation therapy more frequent than in conventional treatment? *Arch Surg* 1989;124:229-34.

14. Ward BA, Miller DL, Frank JA, Dwyer AJ, Simmons JT, Chang R, Shawker TH, Choyke P, Chang AE. Prospective evaluation of hepatic imaging studies in the detection of colorectal metastases: Correlation with surgical findings, Surg 1989;105:180-7.
15. Sindelar WF, Kinsella TJ. Intraoperative radiation therapy for locally advanced cancers, South Med J 1989;82:358-63.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 03811-15 SURG

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Immunotherapy of Animal and Human Cancer

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. A. Rosenberg Chief of Surgery, NCI SURG, NCI  
Others: J. McIntosh (Medical Staff Fellow), J. Mule (Senior Staff Fellow),  
S. Topalian (Senior Staff Fellow), B. Fox (Senior Staff Fellow),  
A. Belldegrun (Visiting Scientist), A. Eisenhalt (Visiting Associate), P.  
Aebersold (Expert), R. Cameron (Medical Staff Fellow), A. Kasid (Senior  
Staff Fellow), J. Weber (Senior Staff Fellow), Yehuda Skornick (Visiting  
Scientist)

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Surgery Branch

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

14

## PROFESSIONAL

8

## OTHER

6

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Attempts are being made to develop new immunotherapeutic techniques for the treatment of advanced cancer. A variety of animal models are being used to test the effects of lymphokine activated killer cells, tumor infiltrating lymphocytes and combinations of lymphokines including interleukin-2, tumor necrosis factor and alpha-interferon in the treatment of experimental animal tumors. Current research is attempting to define the factors necessary for achieving successful adoptive immunotherapy in experimental animal models.

A variety of clinical trials are also in progress exploring the application of new adoptive immunotherapies to patients with advanced cancer. Clinical trials are exploring the value of lymphokine activated killer cells and interleukin-2, high-dose interleukin-2 alone, the combination of alpha-interferon and interleukin-2, the combination of interleukin-2 and tumor necrosis factor, and the value of colony stimulating factors in cancer treatment.

Newer efforts are directed at transducing new genes into tumor infiltrating lymphocytes that can increase their therapeutic effectiveness.

PUBLICATIONS

1. Belldegrün A, Muul LM, Rosenberg SA. Interleukin-2 expanded tumor infiltrating lymphocytes in human renal cell cancer: Isolation, characterization and antitumor activity, *Cancer Res* 1988;48:206-14.
2. Rosenberg SA. The development of new immunologic approaches to cancer therapy, *Blood Purification* 1988;6:69-76.
3. Belldegrün A, Uppenkamp I, Rosenberg SA. Antitumor reactivity of human lymphokine activated killer (LAK) cells against fresh and cultured preparations of renal cell cancer, *J Urol* 1988;139:150-5.
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## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06654-12 SURG

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

## Studies in Malignant Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)

P.I. W. F. Sindelar

Senior Investigator

SURG, NCI

## COOPERATING UNITS (if any)

Others: Radiation Oncology Branch

NCI

## LAB/BRANCH

Surgery Branch

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

1.0

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type Do not exceed the space provided )

Patients with gastrointestinal carcinomas have been studied for evidence of reactivity against tumor-associated determinants expressed on both fresh and cultured syngeneic or allogeneic tumor cells using immunoperoxidase staining techniques. Tumor-associated antigens have been isolated from both animal and human pancreatic cancers and have been investigated for possible applications to immunotherapy or methods of immunodiagnosis. Monoclonal antibodies have been developed to tumor-associated determinants in both hamster and human pancreatic cancers. Tolerance of various normal and surgically-manipulated tissues to intraoperative radiotherapy has been investigated in dogs to determine both acute and long-term toxicity from radiation effects. Clinical trials of intraoperative radiotherapy have been performed including feasibility and developmental studies, randomized trials in resectable and unresectable pancreatic carcinoma, randomized trials in gastric carcinoma, and randomized trials in retroperitoneal sarcomas. Tolerance of normal and surgically-manipulated tissues to photodynamic therapy using hematoporphyrin derivatives and laser light has been investigated in dogs to determine toxicity and to establish dose levels applicable for clinical practice. Clinical trials of intraperitoneal photodynamic therapy have been initiated for the treatment of peritoneal carcinomatosis and peritoneal surface malignancies.

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6. Kinsella TJ, Sindelar WF, Lack E, Glatstein E, Rosenberg SA. Preliminary results of a randomized study of adjuvant radiation therapy in resectable adult retroperitoneal soft tissue sarcomas, *J Clin Oncol* 1988;6:18-25.
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9. Sindelar WF. Intraoperative radiotherapy in carcinoma of the stomach and pancreas. In: Schlag P, Hohenberger P, Metzger U, eds. *Combined Treatment Modalities in Gastrointestinal Tract Cancer*. New York: Springer-Verlag, 1988;226-43.
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11. Sindelar WF, Hoekstra HJ, Kinsella TJ, Barnes M, DeLuca AM, Tochner Z, Pass HI, Kranda KC, Terrill RE. Tolerance of canine esophagus to intraoperative electron beam radiotherapy, *Int J Radiat Oncol Biol Phys*, 1988; 15:663-69.

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15. Sindelar WF. Clinical experience with regional pancreatectomy for adenocarcinoma of the pancreas, Arch Surg 1989;124:127-32.
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## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06657-07 SURG

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies with TNF and Zollinger-Ellison Syndrome

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I. J. A. Norton Senior Investigator SURG, NCI

Others:	D. Fraker	Medical Staff Fellow	SURG, NCI
	B. Sheppard	Medical Staff Fellow	SURG, NCI
	G. Salomon	Expert	SURG, NCI
	C. Buresh	Biologist	SURG, NCI
	C. Jensen	Medical Staff Fellow	SURG, NCI
	H. Langstein	Medical Staff Fellow	SURG, NCI

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Surgery Branch

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

8.0

## PROFESSIONAL:

7.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TNF decreases food intake and body weight gain in rats, but with repeated i.p. administration rats become tolerant to the effects of TNF on food intake and body weight gain. When tolerant rats are given a subsequent inoculation of sarcoma, they live significantly longer than control non-tolerant rats. Rats tolerant to TNF's effects on food intake and body weight are also tolerant to a lethal injection of TNF or endotoxin (LPS). Tolerance to LPS also protects against TNF. However, the mechanisms of tolerance to LPS and TNF are not the same, because LPS tolerance decreases macrophage secretion of TNF and TNF tolerance increases macrophage secretion of TNF. Tolerance can also be achieved by a single iv dose of TNF and it is cross-protective vs LPS, sepsis and TNF. Sarcoma-bearing rats have circulating cachectin activity in their serum and levels increase as food intake decreases, body weight decreases and tumor burden increases. Tumor resection reverses serum cachectin activity in their serum and levels increase as food intake decreases, body weight decreases and tumor burden increases. Tumor resection reverses serum cachectin activity levels. This experiment plus the previous one suggest that TNF is a mediator of cachexia in this rat model. Cachectin/TNF appears to be secreted by host macrophages in TB rats, because studies document that these macrophages have heightened TNF production in response to endotoxin and other stimuli. TNF appears to partially cause the toxicity of IL-2 administration because antibodies to TNF reverse the toxicity of IL-2 and allow more IL-2 to be administered and a greater therapeutic effect.



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2. Fraker DL, Stovroff MC, Merino MJ, Norton JA. Tolerance to TNF in rats and the relationship to endotoxin tolerance and toxicity, J Exp Med 1988;168:95-105.
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7. Sheppard BC, Fraker DL, Norton JA. Prevention and treatment of endotoxin and sepsis lethality with recombinant human TNF, Surgery, in press.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06658-07 SURG

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Effect of Cytokines on Breast Cancer Cell Growth and Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. N. Danforth Senior Investigator SURG, NCI

Others: M. Sgagias IRTA Fellow SURG, NCI

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Surgery Branch

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.0

## PROFESSIONAL:

2.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are studying the effect of cytokines on the growth and metabolism of human breast cancer cells. We have found that interleukin-1 inhibits the growth of hormone-dependent MCF-7 but not hormone independent MDA cells in vitro. The inhibition is dose dependent and does not require the continuous presence of IL-1. Cell cycle analysis suggests IL-1 is blocking cell growth at G<sub>0</sub>/G<sub>1</sub>. IL-1 has also been found to down-regulate the estrogen receptor, but not the progesterone receptor, in these cells over 12-48 hours. This down-regulation of ER is dose-dependent, occurs without a change in the dissociation constant (K<sub>d</sub>), and is not a direct effect of IL-1 on the ER protein. The effect on ER is blocked by cycloheximide and thus requires continuous protein synthesis. When cells are pretreated with IL-1, progesterone receptor synthesis in response to estradiol is enhanced 3 fold, indicating that IL-1 increases estrogen responsiveness of these cells. The effect of IL-1 on gene expression of EGF receptor, PDGF, and TGF beta was studied by Northern blotting in several breast cancer cell lines; no effect of IL-1 at time points of 1 hour to 7 days was noted. Studies of the effect of IL-1 on estradiol stimulation of growth and ER and PR gene expression are in progress. The effect of other cytokines on cell growth have been studied and demonstrated that tumor necrosis factor (TNF), but not IL-2, IL-4, IL-6, or g-interferon inhibit growth of MCF-7 cells. TNF inhibits growth of these cells in a dose dependent manner, and also acts synergistically with IL-1 to inhibit cell growth. Similarly, IL-1 and TNF were found to enhance TNF gene expression by N. blotting. IL-1 plus TNF had a greater stimulatory effect on TNF expression than either cytokine alone, further indicating that IL-1 inhibition of these cells may be via enhanced synthesis/secretion of TNF. Studies of the effect of IL-1 on growth, TNF expression and ER/PR metabolism of solid tumors in vivo, as well as studies of gene expression of cytokines and growth factors in peripheral blood mononuclear cells from normal subjects and breast cancer patients are in progress.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06659-07 SURG

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

## Studies of Urologic Malignancy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.	W. M. Linehan	Head, Urologic Oncology Section	SURG, NCI
Others:	M. M. Walther	Senior Investigator	SURG, NCI
	G. H. Weiss	Medical Staff Fellow	SURG, NCI
	P. Anglard	Visiting Fellow	SURG, NCI
	M. W. Ewing	Biotechnology Fellow	SURG, NCI
	S. C. Liu	Chemist	SURG, NCI
	E. E. Trahan	Medical Technician	SURG, NCI

## COOPERATING UNITS (if any)

Others:	B. Zbar	NCI
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## LAB/BRANCH

Surgery Branch

## SECTION

Urologic Oncology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

6.75

## PROFESSIONAL

4.75

## OTHER

2

## CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

## SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided )

We are studying the molecular genetics of human renal cell carcinoma, evaluating growth factor production by human genitourinary tumors and participating in studies of adoptive immunotherapy in patients with advanced malignancies. By use of molecular techniques we have identified DNA sequence deletions in the short arm of chromosome 3 in both sporadic as well as a familial form of human renal cell carcinoma (RCC). The loss alleles at loci on the short arm of chromosome 3 may indicate the presence of a RCC recessive oncogene at this location. We have also identified DNA sequence deletions at chromosome 11, chromosome 17 and at chromosome 13 at the retinoblastoma locus. We have demonstrated, also in collaboration with Berton Zbar, deletions on chromosome 3 in other tumors associated with familial RCC. We have demonstrated that chromosome 3s retained in the RCC tumors of the probands were inherited from the affected parent, which is consistent with our previous data suggesting that the RCC gene is located on chromosome 3p and that 3p loss may represent the second step of a two-mutation process. The DNA sequence deletions observed at other chromosomal loci (11 and/or 13) may be important in progression or metastasis. We have evaluated patients at risk for familial RCC in order to perform linkage analysis which has demonstrated that the VHL disease gene is located on chromosome 3. In evaluating the effect of suramin on human genitourinary tumors we have found that suramin inhibits proliferation and thymidine incorporation of human prostate carcinoma and that suramin reversibly inhibits the effect of TGF-beta on human RCC in vitro. We are currently evaluating the effect of suramin on growth-factor induced mitogenesis in these cells on the molecular events associated with suramin-inhibition of prostate carcinoma growth. These studies may provide a basis for the development of new therapeutic strategies for treatment of prostate as well as renal cell carcinoma. We also evaluate and participate in treatment of patients with advanced RCC with IL-2 based immunotherapy.

1. Webb DE, Austin HA III, Belldegrün A, Vaughan E, Linehan WM, Rosenberg SA. Metabolic and renal effects of Interleukin-2 immunotherapy for metastatic cancer, *Clin Nephrol* 1988;30:141-5.
2. Linehan WM, Robertson CN, Miller ET, Santora AC. Evaluation of the panacrine and endocrine effects of genitourinary tumor produced growth factors, *Prog Clin Biol Res* 1988;277:135-43.
3. Ozols RF, Ihde D, Linehan WM, Young RC. Management of high risk patients with advanced testis cancer: National Cancer Institute Approach, *Semin Oncol* 1988;15-335-8.
4. Linehan WM. This month in investigative urology. Adoptive immunotherapy of genitourinary tumors with Interleukin-2, *J Urology* 1988;140:838-9.
5. Linehan WM, Anglard P, Miller ET, Merino M, Zbar B. Improved detection of allele loss in human renal cell carcinoma after removal of leukocytes by immune selection, *J Natl Cancer Inst* 1989;81:287-90.
6. Gomella LG, Sargent ER, Linehan WM, Kasid A. Transforming growth factor-beta inhibits the growth of renal cell carcinoma in vitro, *J Urology* 1989;141:1240-4.
7. Belldegrün A, Webb DE, Austin HA, Steinberg SM, Linehan WM, Rosenberg SA. Renal toxicity of Interleukin-2 administration in patients with metastatic renal cell cancer: Effect of pretherapy nephrectomy, *J Urology* 1989;141:499-503.
8. Linehan WM, Shipley W, Longo D: Cancer of the kidney and ureter. In: DeVita VT, Hellman S, Rosenberg SA, eds. *Principles and Practices of Oncology*, Philadelphia: JB Lippincott, 1989;979-1007.
9. Ashby H, Dimattina M, Linehan WM, Robertson CN, Queenan JT, Albertson B. The inhibition of human adrenal steroidogenic enzyme activities by suramin, *J Clin Endocrin Metab* 1989;68:505-10.
10. Elliot S, Striker LJ, Doi T, Linehan Wm, Striker GE. Hepatoma G2 conditioned medium facilitates early outgrowth of endothelial cells from isolated golmeruli, *Kidney International* 1989;35:1245-8.
11. Gomella L, Linehan WM. Bladder cancer. In: Magrath IT, ed. *New Directions in Cancer Treatment*. New York: Springer Verlag, 1989;496-500.
12. Robertson CN, Linehan WM. Renal Cell Carcinoma. In: Magrath IT, ed. *New Directions in Cancer Treatment*. New York, Springer Verlag, 1989;468-72.
13. Linehan WM, Robertson CN, Rosenberg SA. Clinical experience with adoptive immunotherapy in patients with renal cell carcinoma, *Urology Annual* Norwalk, Appleton, 1989;131-8.

14. Tory K, Brauch H, Linehan WM, Oldfield E, Barba D, Filling-Katz M, Nakamura Y, White R, Seizinger B, Lerman M, Zbar B. Common chromosome 3p deletion in three tumor types associated with Von Hippel Lindau Disease, *J Natl Cancer Inst*, in press.
15. Rosenberg SA, Lotze MT, Yang JC, Aebersold PM, Linehan WM, Seipp CA, White DE. Experience with the use of high dose Interleukin-2 in the treatment of 652 patients with cancer, *Annals of Surgery*, in press.
16. Linehan WM, Robertson CN, Anglard L, Gomella LG, Sargent ER, Wade T, Kasid A. Clinical Perspective: Renal Cell Carcinoma-Potential Biologic and Molecular Approaches to Diagnosis and Therapy, *Cold Spring Harbor, Lab Cancer Cells* 7, in press.
17. Haas GP, Pittaluga S, Gomella L, Travis WD, Sherins RJ, Doppman JL, Linehan WM, Robertson CN. Clinically occult leydig cell tumor presenting with gynecomastia, *J Urology*, in press.
18. Horan JJ, Robertson CN, Cary N, Choyke, Peter L, Frank JA, Miller DL, Pass HI, Linehan WM. The Detection of Renal Carcinoma Extension into the Renal Vein and Inferior Vena Cava: A Prospective Comparison of Venacavography and MRI, *Journal of Urology*, in press.
19. Linehan WM, Robertson CN, Rosenberg SA. Adoptive immunotherapy of renal cell carcinoma using lymphokine activated killer cells and recombinant Interleukin-2. In: Williams RD, ed. *Advances in Urologic Oncology*. New York: MacMillan Publishing Company 1989, in press.
20. Ozols RF, Ihde DC, Linehan WM, Young RC. High dose chemotherapy regimen for treatment of poor prognosis nonseminomatous germ cell tumors. In: Johnson DE, Logothitis C, Von Eschenbach AC, eds. *Systemic Therapy for Genitourinary Cancers*, Yearbook Medical Chicago 1989, in press.
21. Keiser HR, Doppman JL, Robertson CN, Linehan WM, Averbuch SD. Diagnosis, localizations and management pheochromocytoma. In: Lack EE, ed. *Pathology of the Adrenal Gland*. New York: Churchill Livingston Inc., 1989, in press.
22. Agodora LYC, Striker LJ, Robertson CN, Linehan WM, Striker GE. Glomerular lesions in neoplasia, *Am J Kid Dis*, in press.
23. Linehan WM. Thoracoabdominal Radical Nephrectomy, In Glenn JF, ed. *Urologic Surgery*, Philadelphia: JB Lippincott, in press.
24. Gomella LG, Sargent ER, Anglard P, Linehan WM, Kasid A. Overexpression of epidermal growth factor receptor gene in human renal cell carcinoma tissues, *Surgical Forum* 1988, in press.

25. Wade TP, Gomella LG, Sargent ER, Anglard P, Kasid A, Linehan WM.  
Southern blot analysis of transforming growth factors alpha and  
beta DNA in normal kidney and renal cell carcinoma tissue,  
Current Surgery, in press.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

The Study of Interleukin-2 Based Immunotherapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. C. Yang Senior Investigator SURG, NCI

Others: D. Perry-Lalley Microbiologist SURG, NCI

K. Griffith Medical Staff Fellow SURG, NCI

E. Shlasko Medical Staff Fellow SURG, NCI

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Surgery Branch

## SECTION

Tumor Immunology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

4.0

## PROFESSIONAL

3.0

## OTHER

3.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our laboratory is attempting to improve the therapeutic efficacy of tumor infiltrating lymphocytes (TIL) in murine cancer models. We have developed a method for separating TIL from tumors using antibody-coated magnetic beads. Selective culture conditions were also developed in order to maintain and improve the therapeutic efficacy of these separated TIL. This methodology enabled us to produce effective TILs from a non-immunogenic tumor. Additionally, we have been studying the in vivo traffic of both murine and human TIL. We have recently published a paper describing specific traffic of TIL to sites of tumor in patients with melanoma. Studies on the toxicity generated by IL-2 have focused on the production of leukotrienes. We recently demonstrated an increase in the stimulated production of leukotrienes by lymphocytes pre-treated with interleukin-2. These investigations are continuing in the murine model to demonstrate whether or not leukotrienes play a role in the vascular leak syndrome seen with interleukin-2. Other current investigations involve the use of immunohistochemistry for identifying the production of lymphokines at the sites of tumor regression in response to TIL therapy. This is in an attempt to identify mediators of anti-tumor responses when immunotherapy is given. Another effort is focused on the role of lymphocyte motility and chemotaxis in the anti-tumor response of TIL. TIL are being separated on the basis of motility in an effort to isolate populations with the increased anti-tumor efficacy. In addition, stimulators of motility and mediators of chemotaxis are being employed in both in vitro and in vivo models.

PUBLICATIONS

Z01 CM 06660-06 SURG

1. Spiess PJ, Yang JC, Rosenberg SA. In vivo antitumor activity of tumor-infiltrating lymphocytes expanded in recombinant interleukin-2, JNCI 1987;5:79.
2. Papa MZ, Yang JC, Vetto JT, Shiloni E, Eisenthal A, Rosenberg SA. Combined effects of chemotherapy and interleukin 2 in the therapy of mice with advanced pulmonary tumors, Cancer Res 1988;48:122-9.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06661-06 SURG

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Immunologic Studies in Patients with Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	M.T. Lotze	Senior Investigator	SURG, NCI
Others:	M.C. Custer	Microbiologist	SURG, NCI
	D. Jablons	Medical Staff Fellow	SURG, NCI
	J. Rubin	Staff Fellow	SURG, NCI
	Y. Kawakami	Visiting Fellow	SURG, NCI
	H. Stotter	Visiting Associate	SURG, NCI
	M. Tran	Stay In School Student	SURG, NCI

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Surgery Branch

## SECTION

Tumor Immunology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

4.5

## PROFESSIONAL:

3.5

## OTHER

1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major goal of our laboratory is to develop and evaluate immunologic reagents in the therapy of patients with cancer. In patients receiving IL-2 base therapy, we have carefully evaluated the immunohistologic appearance of responding and non-responding lesions in demonstrated correlation between response in T cell infiltrate. In most lesions with T cell infiltrate, there appears to be a close correlation with DR expression both on the infiltrating T cells as well as tumor cells. We have shown that a variety of cytokines are present in the serum of the patients receiving IL-2 and have further shown that patients receiving IL-2 have decreased ability of PMN to respond to PMA with a respiratory burst and migration. In addition to being found in the serum of patients receiving IL-2 and TNF; IL-6 can also be demonstrated in the serum of mice with progressively growing tumors with a variety of different histologies. We have shown that gamma interferon and tumor necrosis factor pre-treatment of the target cells causes markedly enhanced susceptibility to lysis by tumor infiltrating lymphocytes. This may have relevance in the development of clinical protocols. We have shown previously that IL-4 promotes the growth of melanoma TIL's as well as suppresses the generation of lymphokine activated killer cells. We have examined the ability of IL-4 to synergize with IL-2 in expanding TIL's from other malignancies. We have evaluated the role of MCSF in enhancing monocyte mediated ADCC with gamma interferon. In addition we have demonstrated enhanced ADCC mediated by cells obtained from patients receiving IL-2 in the context of our monoclonal antibody protocols. We have evaluated a series of 19 patients receiving IL-4 for acute immunologic effects and developed a rapid fluorescent assay for detection of IL-4 in serum. We have established that IL-4 has a short half life in humans with an alpha clearance of approximately 8 minutes and a beta clearance of 30 to 60 minutes.

1. Kawakami Y, Rosenberg SA, Lotze MT. Interleukin-4 promotes the growth of tumor infiltrating lymphocytes specific for human autologous melanoma, *J Exp Med* 1988;168:2183-91.
2. Rosenberg SA, Packard B, Aebersold PM, Solomon D, Topalian SL, Toy S, Simon P, Lotze MT, Yang JC, Seipp CA, Simpson C, Carter C, Bock S, Schwartzentruber D, Wei JP, White DE. Use of tumor Infiltrating lymphocytes and Interleukin-2 in the immunotherapy of patients with metastatic melanoma: A Preliminary Report, *N Engl J Med* 1988;319:1676-80.
3. Jablons DM, Mule JJ, McIntosh JK, Sehgal PB, May LT, Huang CM, Rosenberg SA, Lotze MT. Interleukin-6/Interferon B-2 as a circulating hormone: Induction by cytokine administration in humans, *J Immunol* 1989;142:1542-7.
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5. Rosenberg SA, Longo DL, Lotze MT. Principles of biologic therapy. In: DeVita V, Hellman S, Rosenberg SA, eds. Principles and Practices of Oncology. Philadelphia: JB Lippincott, 1989;301-47.
6. Del Vecchio S, Reynolds JC, Carrasquillo JA, Blasberg RG, Neumann R, Lotze MT, Bryant GJ, Parkas RJ, Larson SM. Local distribution and concentration of  $^{131}\text{I}$ -9.2.27 monoclonal antibody in malignant melanoma following intravenous injection, *Cancer Res*, in press.
7. Kawakami Y, Custer MC, Rosenberg SA, Lotze MT. Interleukin-4 regulates Interleukin-2 induction of lymphokine activated killer activity from human lymphocytes, *J Immunology*, in press.
8. Sakahara H, Reynolds JC, Carrasquillo JA, Lora ME, Maloney PJ, Larson SM, Neumann RD, Lotze MT. In vitro complex formation and biodistribution of mouse antitumor monoclonal antibody in cancer patients, *J Nucl Med*, in press.
9. Rosenberg SA, Lotze MT, Aebersold PM, Yang JC, Chang AE, Avis FP, Bock SN, Schwartzentruber D, Wei JP, Leitman S, Linehan WM, Robertson CN, Seipp CA, Simpson CG, White DE, Steinberg SM. Prospective randomized trial of high dose Interleukin-2 alone or with lymphokine activated killer cells for the treatment of patients with advanced cancer, *J Clin Oncol*, in press.
10. Denicoff KD, Durkin TM, Lotze MT, Quinlan PE, Davis CL, Listwak SJ, Rosenberg SA, Rubinow DR. The neuroendocrine effects of Interleukin-2 treatment, *J Clin Endocrine and Metabolism* 1989, in press.

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12. Jablons DM, McIntosh JK, Mule JJ, Nordan JJ, Rudikoff S, Lotze MT. Induction of Interferon- $\beta_2$ / Interleukin-6 (IL-6) by cytokine administration and detection of circulating IL-6 in the tumor-bearing state In: Sehgal PB, ed. Regulation of the Acute Phase and Immune Responses: A New Cytokine. New York Academy of Sciences: Grieninger G, Tosato G, 1989, in press.
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14. Lotze MT, Kawakami Y, Rosenberg SA. Immunotherapy Proctotols at the National Cancer Institute. In: Bertoglio J, Fradelizi D, eds. Current Status and Future Prospects. Lymphokines and lymphokine receptors, 1989, in press.
15. Fisher B, Keenan A, Garra BS, Steinberg SM, White DE, DeBisceglie AM, Hoofnagle JH, Yolles P, Rosenberg SA, Lotze MT. Interleukin-2 induces profound reversible cholestasis: A detailed analysis in treated patients, J Clin Oncology, in press.
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## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06662-03 SURG

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies of Phototherapy for Thoracic Malignancies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. H. I. Pass Senior Investigator SURG, NCI

Others: W. Matthews Chemist SURG, NCI  
 R. Perry Medical Staff Fellow SURG, NCI  
 S. Evans Medical Staff Fellow SURG, NCI

## COOPERATING UNITS (if any)

Others: J. Mitchell Deputy Branch Chief ROB, NCI  
 A. Russo Head, Experimental Phototherapy Section ROB, NCI

## LAB/BRANCH

Surgery Branch

## SECTION

Thoracic Oncology Section

## INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

4.0

## PROFESSIONAL

3.0

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our laboratory has continued investigations of photodynamic therapy for the treatment of thoracic malignancies by sensitization of malignant cells with dihematoporphyrin ether followed by illumination with 630 nm light. Since October of 1988 we have established that there is a dose rate effect for photodynamic therapy of lung cancer cells. We have published our results regarding the use of intralipid as light diffusing media for the treatment of cells by photodynamic therapy showing there is an optimal concentration of intralipid for maximal cytotoxicity via light scattering effects. These data have great implications for the treatment of intracavitary disease. We have completed a project in which we have used a phantom model of the thoracic cavity and diode systems to show the delivery of PDT to the thoracic cavity can be imparted. We have now characterized the photodynamic therapy efficacy in vitro of 6 established human lung cancer lines and one human normal lung fibroblast line. We found that adenocarcinoma is the least sensitive to PDT while the human fibroblast is the most sensitive. There are significant differences between phototherapy effects among the other lines. We have completed a project which has definitely shown that the sensitizer DHE can be delivered to cells in vitro by "doping" the sensitizer with low-density lipoprotein. The delivery of the DHE with LDL is via saturation kinetics and the significant differences between the lines correspond to their in vitro phototherapy efficacy. Therapy of murine macrophages elicited by thioglycolate will cause these macrophages to start production of tumor necrosis factor. These data represent the first demonstration of cytokine release due to photodynamic therapy and may explain indirect cytotoxicity of PDT as well as vascular effects. We have now treated 13 patients with endobronchial disease with PDT. Approximately 15 patients have been treated with recurrent or varying carcinomatosis with intraabdominal PDT.

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2. Delaney T, Russo A, et al. Bronchoscopic phototherapy at comparable dose rates: Early results, Ann of Thor Surg 1989;47:693-9.
3. Matthews W, Cook J, Mitchell JB, et al. In vitro photodynamic therapy of human lung cancer: Investigation of dose-rate effects, Cancer Research, 1989;49:1718-21.
4. Perry R, Evans S, Matthews W, et al. Potentiation of phototherapy cytotoxicity with light scattering media, J Surg Res 1989;46:386-90.
5. Perry R, Evans S, Smith P, et al. Dosimetry parameters in phantom model PDT. Lasers in Surgery and Medicine 1989;143(suppl 1).
6. Pass HI, Delaney T, Glatstein E., et al. Photodynamic therapy. In: Wiernak PH, ed. Mediguide to Oncology, 1989, in press.



SUMMARY REPORT  
ASSOCIATE DIRECTOR FOR THE RADIATION RESEARCH PROGRAM  
DIVISION OF CANCER TREATMENT  
NATIONAL CANCER INSTITUTE  
OCTOBER 1, 1988 - SEPTEMBER 30, 1989

I. INTRODUCTION

In 1982 the Radiation Research Program (RRP) was established in the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health (NIH). It is an extramural program that has two Branches: the Diagnostic Imaging Research Branch (DIRB) and the Radiotherapy Development Branch (RDB). The scientific mission of the Radiation Research Program is to develop research where radiation and related forms of energy are used in the diagnosis, staging, treatment and post-treatment evaluation of the patient with cancer. Included in the mission of the Radiation Research Program is the planning, development, administration, and evaluation of an extramural radiation research program. This is accomplished by establishing program priorities, allocating resources, maintaining project integration, evaluating program effectiveness, and representing the program in the management and scientific decision making processes of the National Cancer Institute.

For scientific and administrative direction, the RRP relies heavily on the advice of the DCT Board of Scientific Counselors. The Program coordinates research activities with related programs at NCI and NIH, with other Federal agencies, and with national and international research organizations. The RRP provides a radiation research focal point for national and international extramural investigators.

II. PERSONNEL

A. Staffing

1. Office of the Associate Director

John E. Antoine, M.D., Associate Director  
Ruthie S. Herrington, Secretary to the Associate Director  
Wendy R. Fredericks, Biologist  
Richard V. Stepney, Computer Specialist  
Vacant, Clerk-Typist

2. Administrative Office

James Stoneman, Administrative Officer  
Cynthia Glagola, Administrative Technician

3. Diagnostic Imaging Research Branch

Matti Al-Aish, Ph.D., Acting Chief  
Roger Powell, Program Director  
Arlyce Peterson, Branch Secretary

4. Radiotherapy Development Branch

Francis Mahoney, Ph.D., Acting Chief

Thomas Strike, Ph.D., Program Director  
Sandra Zink, Ph.D., Cancer Expert  
Cathy Bailey, Branch Secretary

B. Recruitments

Chief, Diagnostic Imaging Research Branch  
Chief, Radiotherapy Development Branch  
Radiation Oncologist, Radiotherapy Development Branch  
Program Director, Radiotherapy Development Branch

III. MAJOR ACTIVITIES

The Radiation Research Program continues to stimulate, develop, administer and evaluate basic science and clinical research areas in radiation therapy, nuclear medicine, diagnostic imaging, and their related subspecialty areas. At the October 6-7, 1988, Division of Cancer Treatment Board of Scientific Counselors meeting, a Radiation Research Program review was carried out.

The following areas were highlighted:

- (1) A high priority project of the Radiotherapy Development Branch, the neutron clinical trials program, was again reviewed. After many years of planning and development, Phase III trials have been designed and are successfully being carried out. The institutions participating in these trials continue to be the University of Washington, Seattle, Washington; UCLA, Los Angeles, California; and the University of Texas Cancer Center, M. D. Anderson Hospital, Houston, Texas. The efficacy of neutron beam therapy for the treatment of salivary gland tumors has been demonstrated. Data continue to be obtained from clinical trials in the treatment of localized prostate cancer, head and neck tumors, and radio resistant neoplasms. Unfortunately, the cyclotron at M. D. Anderson Hospital, Houston, Texas, experienced a breakdown; however, this has been corrected, and neutron clinical trials have resumed at that institution.
- (2) Continued excellent results in the control of clival chordomas, base of skull chondrosarcomas and uveal melanomas are reported by the proton beam research team at the Harvard Cyclotron Laboratory, Cambridge, Massachusetts. This research team requested permission to submit a grant proposal to DCT, NCI for partial funding of a dedicated hospital based proton clinical research facility. Dr. Chabner appointed a subcommittee chaired by Dr. James Cox to evaluate the proposal, perform a site visit of the Cambridge, Massachusetts, facility and report back to the DCT BSC.

Because of encouraging results there is increasing radiation oncology interest in the use of proton beams for the treatment of malignant disease. A dedicated clinical proton research and treatment unit is under development at the Loma Linda Medical Center in Riverside, California. Interest in proton beam therapy is increasing not only in the United States of America but also in the international radiation research community.

Data from the Heavy Ion Project at the Lawrence Berkeley Laboratory in Berkeley, California, are consistent with data being obtained from the Harvard Cyclotron Proton Beam Project. The heavy ion beam and the proton beam



projects demonstrate that there is a definite place for this type of precision radiotherapy in the treatment of well-defined localized cancers.

- ( 3 ) Intraoperative radiation therapy continues to be clinically investigated and appears to be effective in the treatment of advanced local gynecological rectal tumors, retroperitoneal sarcomas and gastric cancers. Phase II and III Clinical Trials are being performed.
- ( 4 ) Radiation modifiers: The radiation sensitizer contracts of RRP continue to identify and develop substances with radiosensitizing properties. Encouraging results with SR-2508 have been followed by Phase II and Phase III Clinical Trials presently being carried out by the Radiation Therapy Oncology Group (RTOG), and other cooperative groups. The sensitizer program is being reevaluated and there is hope that a large scale automated radiation sensitizer screening program can be developed. The expertise, experience and advice of the Developmental Therapeutics Program is being utilized for the potential development of a new screening program.

The radioprotector, WR-2721, continues to show a protective effect, not only when used with radiation, but also with chemotherapy. Encouraging clinical results are being reported in the use of WR-2721 with chemotherapeutic agents. Larger doses of chemotherapy can be given with the normal tissues protected. Improved therapeutic ratios are being reported but this observation requires substantiation by further clinical investigation.

- ( 5 ) Hyperthermia continues to show promise in the management of malignant disease. In addition to being used with radiation for the improved control of local tumors it is being investigated as an adjunct to chemotherapy in the treatment of systemic disease. However, in the treatment of localized neoplasms thermometry remains an invasive procedure requiring multiple probes be inserted into the patient's tumor. Magnetic Resonance Imaging techniques may make non-invasive thermometry a reality. Persistent difficulties with adequate local deep heating may be overcome by the use of ultrasound techniques. A RRP workshop addressing these problems was held May 12 - 13, 1988. A Recommendation that NCI hyperthermia efforts be coordinated largely through RTOG were made to RRP by the participants of this workshop and this advice has been followed by the Program during 1988 and 1989.
- ( 6 ) The exciting field of photodynamic therapy is a research field in which systemically administered tumor seeking light sensitive compounds are used in conjunction with activating wave lengths of light, usually generated by a laser. Improvements in the light sensitizing compounds are being made and several new compounds are now entering the Decision Network of DCT, NCI. Potential for the treatment of closed space neoplasms such as carcinoma of the ovary, mesothelioma and bladder cancer are being explored. The effectiveness of this therapy in the reestablishment of airway in totally occluded bronchi from lung cancer has been demonstrated. Hopefully, this research area will mature into a treatment modality which will give improved results in the treatment of tumors which commonly recur following conventional therapy , e.g., ovarian cancer. Industry-supported Phase III clinical trials are now being performed for lung, esophageal, and bladder cancer. A photodynamic therapy workshop is planned for the fall of 1989.

- ( 7 ) Dosimetry studies: Research in determining optimal radiation treatment planning is ongoing. These activities include the dosimetry of interstitial radiation therapy, x-ray, and electron and particle beams. Research in radionuclide conjugate dosimetry is extremely important as this therapeutic approach is experiencing rapid growth. An RRP Request for Application (RFA) for Radionuclide Dosimetry received many excellent grant applications of which several will be funded.
- ( 8 ) A new "Patterns of Care" study to evaluate the radiation therapy in the United States is being funded. This retrospective analysis will evaluate data from patients treated for breast, cervix, prostate, and recto-sigmoid cancer and Hodgkins Disease. Early patterns of care studies have proven helpful in identifying methods for the improvement of patient treatment such as in the treatment of prostate cancer, cervix cancer, and Hodgkin's Disease.
- ( 9 ) The rapidly emerging research area of medical informatics, also known as "expert systems", is being exploited in the optimization of radiation treatment and planning. Dr. Henry Swett, Clinical Director of the Department of Radiology at Yale University presented a thorough review of advanced computing in Radiology to the DCT BSC. A Request for Proposal (RFP) has been funded for the development of a system to rapidly and automatically extract anatomic features from diagnostic images, define and delineate tumors from normal tissues, define treatment volumes from tumor contours, optimize treatment plans, display three-dimensional images rapidly and interactively, and improve simulation verification. The impact of medical informatics on the field of radiation oncology and the radiologic sciences will be great, and an even greater impact on the field of medicine in the near future is anticipated.
- ( 10 ) Boron Neutron Capture Therapy (BNCT) is a therapeutic method having the potential for achieving tissue and cell specific radiation therapy which is a major goal of the Radiation Research Program. When a boron compound is deposited in a tumor and the boron excited by low energy neutrons, a subsequent nuclear disintegration of the boron atom results in the release of focal radiation. Early BNCT trials in the treatment of malignant gliomas were carried out in the United States in the 1950s and 1960s but were discontinued because of unacceptable normal tissue side effects. However, Dr. Hatanaka, a neurosurgeon in Japan, has continued using BNCT for the treatment of patients with malignant brain tumors. A Japanese dermatologist, Dr. Mishima, has used 10 borono-phenylalanine to study melanoma in an animal model (pig). He is beginning clinical trials in the use of BNCT for patients who have melanoma.

A workshop on "Boron Compounds Suitable for Neutron Capture Therapy for the Treatment of Cancer" was held May 3-4, 1988, in Annapolis, Maryland. A workshop report was given at the June 1988 DCT BSC by Dr. Albert Soloway which recommended research in the development of improved BNCT compounds be supported.

- ( 11 ) The exciting area of Radioimmunotherapy (RIT) also called Systemic Radiation Therapy (SRT) was again discussed. A RFA passed by the Board of Scientific Counselors for the establishment of a research group of investigators to develop the optimal dosimetry of this rapidly emerging therapeutic technique was released in October 1988 and several grant applications will be funded in FY 89.

In diagnostic imaging, several research areas were reviewed.

- ( 1 ) Diagnostic ultrasound has the potential for tissue characterization which has not been totally exploited. Program emphasis will be placed on making tissue specific diagnosis using non-invasive techniques possible. Hopefully, ultrasound will be helpful in accomplishing this goal. This potential exists in the evaluation of breast disease where ultrasonographic techniques can easily be used.
- ( 2 ) Of the rapidly evolving imaging modalities, none is more dynamic than that of magnetic resonance imaging (MRI) and spectroscopy (MRS). Contracts supported by the Radiation Research Program in the comparison of magnetic resonance imaging and computerized tomography resulted in data that were used in a NIH Consensus Conference on MRI held October 26-28, 1987. A workshop on the use of MRI/MRS for the evaluation of tumors before, during and following therapy is planned.
- ( 3 ) Another goal of the Diagnostic Imaging Research Branch of the Radiation Research Program is to develop the anatomic and functional diagnosis of neoplasms employing single and multiple modality imaging related technology. An RFA dedicated to this purpose was released in 1988. RRP supports grants dealing with the imaging modalities of CT, MRI, MRS, and others. In radionuclide technology exciting research areas include Single Photon Emission Computed Tomography (SPECT), Positron Emission Tomography (PET), and Radionuclide Conjugate Diagnosis.
- ( 4 ) PET scans have been extremely helpful in obtaining functional and anatomic information. Biologically active substances such as Carbon 11, Nitrogen 13, Oxygen 15, and Fluorine 18, have been used to obtain information differentiating tumor tissue from normal tissue. This technology appears to make possible the differentiation of normal brain tissue from brain tumors and the determination of tumor viability following therapy such as radiation therapy. These findings require further investigation and verification.
- ( 5 ) Three dimensional anatomic diagnosis is made possible by the use of Single Photon Emission Computed Tomography (SPECT) units. It is anticipated that this will be an invaluable tool, not only for tumor identification and localization, but may be useful in treatment planning. A workshop on the utilization of SPECT in neoplastic disease is planned.
- ( 6 ) Perhaps the most exciting of the rapidly evolving radionuclide related research areas is that of radiolabeled ligands for use in tumor identification and treatment. The possibility of tumor specific radiodiagnosis is made possible by this technique. An RFA titled "Application of Chelate Conjugated Radiolabeled Monoclonal Antibodies Specifically to Diagnostic Imaging" was released in 1987 and several grants received in response are being funded.

At the February 18-19, 1989, DCT BSC, Dr. Antoine once again reviewed the neutron therapy clinical trials including history and development. The fact that the technical problems of developing cyclotrons for neutron generation were overcome and that Phase III clinical trials are being performed by the Neutron Therapy Working Group was again emphasized. It was noted that there is also increasing interest in neutron therapy clinical trials by the international radiation research community. These Investigators are from as far away as Seoul, Korea, and Capetown, South Africa.

The subcommittee chaired by Dr. William Hendee for the evaluation of the neutron clinical trials made its report at the June 1988 DCT BSC Meeting. The subcommittee felt that the scientific questions asked in the Phase III protocols should be answered. This should be done in as expeditious and financially responsible manner as possible. It was realized that some of the clinical trials would extend beyond the 1989 maturation date of the neutron contracts, and that mechanisms should be developed to complete the high priority Phase III trials in prostate, head and neck and lung cancer. This recommendation is consistent with the position taken by the Radiation Research Program over the past several years. The remaining Phase III trials will be completed by 1992 with decreasing NCI financial support planned.

Dr. Antoine discussed the Radiologic Diagnostic Oncology Group (RDOG). The principal investigator of this Group is Barbara J. McNeil, M.D., Ph.D., Head of the Department, Health Care Policy, Professor of Radiology, Brigham and Women's Hospital, Boston, Massachusetts. These are the first prospective Diagnostic Imaging trials designed to study oncologic disease. RDOG-I is studying prostate and lung cancer. The Board of Scientific Counselors gave approval to RDOG II in a prior meeting of the DCT BSC. It will study pancreatic and colorectal cancer.

Dr. Antoine introduced Dr. McNeil who gave a thorough presentation of the methodology and accomplishments of RDOG I. Following this, Dr. Antoine requested approval from the Board of Scientific Counselors for the study of musculoskeletal and head and neck tumors (RDOG III) utilizing the same methodology of RDOG I and II. Approval was given.

Dr. Antoine then discussed the utilization of PET scanning in the study of brain tumors. Pioneering work by Dr. Di Chiro at the NIH and other investigators has resulted in data which support the thesis that PET scanning is able to determine brain tumor prognosis, extent, and viability following treatment. Dr. Antoine requested approval of scientific concept for the use of PET scanning in the study of brain tumor metabolism before, during and following therapy. Approval was given by the Board of Scientific Counselors.

Dr. Antoine then discussed utilization of diagnostic imaging techniques in the study of seropositive asymptomatic AIDS patients. At the present time it is not exactly known when drug therapy should be instituted in the seropositive asymptomatic AIDS patients. Psychometric testing has been used to determine early cerebral involvement. The possibility of using imaging techniques such as MRI and PET scanning to detect early involvement of the brain was discussed. Dr. Antoine presented several brain scans of patients with cerebral involvement which demonstrated improvement following the instigation of drug therapy. Dr. Antoine then requested approval for the scientific concept of clinical diagnostic studies of AIDS affected brain using PET and other imaging modalities. This scientific concept would be developed as a RFA. The Board of Scientific Counselors deferred approval until further scientific advice was obtained from an NIH AIDS advisory group.

Following this, Dr. Antoine introduced Dr. Herman Suit, Chairman of the Department of Radiation Medicine, at the Massachusetts General Hospital (MGH), Boston, Massachusetts, who gave a presentation about the Proton Beam Research Project centered at the Harvard Cyclotron Laboratory in Cambridge, Massachusetts. This was followed by a site visit report from Dr. James Cox, Chairman of an Ad Hoc Advisory Committee to the DCT BSC on the proposed

dedicated proton beam project to be located at the MGH. The Proton Beam Research Project and the proposal were further discussed in closed session of the DCT BSC.

Dr. Antoine introduced Dr. Dougherty of Roswell Park Hospital in Buffalo, New York, who presented the results of scientific work in Photodynamic Therapy (PDT). This was followed by a presentation by Dr. Eli Glatstein and Dr. Harvey Pass both from the Intramural Program, describing their research.

Dr. Antoine emphasized collaborative efforts of the Extramural and Intramural Programs are made in areas of mutual scientific interest. He also noted that the RRP has a close working relationship with the Intersociety Commission for Radiation Oncology (ISRO), and this Group has identified PDT as a high priority research area. A RRP sponsored workshop on Photodynamic Therapy is planned for the near future.

At the June 5-6, 1989, DCT, BSC, Meeting Dr. Antoine introduced Martin Brown, Ph.D., Professor of Radiation Oncology and Director of the Division of Radiobiology at Stanford University, Stanford, California. Dr. Brown gave a thorough and stimulating review of the radiation sensitizer field with emphasis on the sensitizers produced by the current radiation sensitizer synthesis contract.

A number of methods have been investigated to overcome the resistance of hypoxic tumor cells to radiation, e.g., high LET radiation, hyperbaric oxygen, and oxygen mimicking radiosensitizers. The radiosensitizers which demonstrated promise and were introduced into the clinic were from the chemical class called nitroimidazoles. The nitroimidazoles are from the category of electron affinic, hypoxic cell sensitizers. A product of this contract, SR 2508, is a radiation sensitizer which is now in Phase III Trials both in the United States (RTOG) and in Europe. At the present time, SR 2508 represents the optimal compound of the nitroimidazole class. Other compounds which have come from this contract include SR-4233, a specific hypoxic cell cytotoxic agent, and BSO originally tested as a radiosensitizer which has now been introduced into the clinic as a chemosensitizer.

Using the principles and approaches learned from the systemic study of the nitroimidazole and the non-nitro compounds investigated thus far, future efforts will be directed toward the optimization of other classes of chemical compounds which show activity in the radiosensitizer stream. Emphasis will be placed on the rational design and development of compounds with and without the nitro group which have different mechanisms of radiosensitizing action, i.e., hypoxic cell sensitizers, inhibitors of potential lethal damage, glutathione depleters, and shoulder modifiers.

Following discussions of the radiosensitizers field, Dr. Antoine asked for Board approval for the recompetition of the radiosensitizer synthesis contract. Approval was unanimously given by the Board of Scientific Counselors.

Following recompetition of the radiosensitizers synthesis contract, Dr. Antoine presented the scientific concept, "Clinical Diagnostic Studies of Aids Affected Organs Using PET and Other Imaging Modalities." Diagnostic imaging techniques will be used in the seropositive but asymptomatic patient to determine when drug therapy should be initiated and to evaluate the results of therapy. This scientific concept will be developed as an RFA. At the previous Board of Scientific Counselors Meeting, the Board requested that additional advice be obtained from experts in the field for use in the development of this scientific concept. Dr. Antoine reported that a meeting with Drs. Pizzo, Feuerstein, and Yarchoan, all experts in the AIDS field, had taken place. This advisory group also recommended that the RFA be developed but that in

addition to imaging studies that other diagnostic testing with emphasis on psychometrics be included. This advisory group recommended that the initial organ site for study should be the brain. Dr. Broder also had given his support to this scientific concept. Following an in-depth discussion of the scientific merits of this concept the Board of Scientific Counselors approved the concept. This will be funded with AIDS funds allocated to the NCI grant pool.

Dr. Antoine then informed the BSC that a workshop had been held on the use of proton beam and small field precision radiation therapy April 27-29, 1989, in Bethesda, Maryland. Also, a workshop for the improvement of the Radiation Sensitizer Screening Program was held May 11-12, 1989 in Bethesda, Maryland. Recommendations of these workshops will be available in the near future. A Photodynamic Therapy workshop is planned for the Fall of 1989.

Dr. Antoine then updated the BSC on the Fast Neutron Beam Project. Both UCLA and University of Washington continue to accrue patients as anticipated. M.D. Anderson's cyclotron returned to function in May of 1989 and patients are being treated. Maximal efforts to obtain third party reimbursement are being made at all institutions. Dr. Antoine concluded his remarks about the fast neutron project by stating that the funding formula developed by the DCT Ad Hoc Committee chaired by Dr. William Hendee was being followed. It is anticipated that all Phase III Trials can be completed by 1993.

The Radiation Research Program being a completely extramural program uses the scientific members of its extramural community for the development of research projects. This advice is obtained at scientific meetings, from representative members of the Board of Scientific Counselors, and from the use of workshops in promising research areas. Further information on the Radiation Research Program's workshops and scientific initiatives will be given in the reports from the Radiotherapy Development Branch and the Diagnostic Imaging Research Branch, RRP, DCT, NCI.

#### IV. SCIENTIFIC OVERVIEW

##### A. DIAGNOSTIC IMAGING RESEARCH BRANCH

The Diagnostic Imaging Research Branch (DIRB) RRP, DCT, NCI continues to develop and administer basic and clinical diagnostic imaging research using both ionizing and nonionizing radiations. Areas of research include nuclear magnetic resonance imaging (MRI), and spectroscopy (MRS), as well as NMR microscopy, computerized tomography (CT), ultrasound, or x-ray diagnosis and instrumentation, and image perception. Other research areas include digital radiography, methods of acquiring , sorting, viewing and communicating diagnostic imaging, and (PACS). The growth of DIRB continues to be satisfactory. Starting with a modest budget of \$3.5 million in 1982, the DIRB budget has grown to an estimated \$36.5 million in 1989.

Magnetic resonance imaging/spectroscopy and nuclear medicine research continue to be the two major areas of funding at DIRB. Areas of increasing interest and significance are the use of monoclonal antibodies in imaging and the collaborative clinical diagnostic imaging research. The following is a summary of DIRB actual budget FY88 and estimated budget FY89.

##### FY88 AND 89 BUDGETS

	\$ (in thousands)			
<u>GRANTS</u>	<u>FY88</u>	<u>FY89</u>	<u>FY88</u>	<u>FY89</u>
Coop. Agree.(RDOG I) (U01, RFA 86-CA-10)	6	7	710	1,189
Coop. Agree. (RDOG II) (U01, RFA 88-CA-02)	0	5	0	825
Traditional (R01)	111	99	21,043	19,848
Program Projects (P01)	8	9	7,936	9,186
Conf. & New Investigator (R13 & R23)	6	4	145	102
SBIR*	14	16	2,393	2,934
First Awards	4	7	397	620
RFA, 87-CA-33	2	2	201	225
RFA, 87- CA-36	2	2	302	310
RFA, 87-CA-20 (OSP)	3	3	368	370
RFA, 88-CA-10	0	3	0	400
<u>TOTAL GRANTS</u>	156	157	33,495	36,009
<u>CONTRACTS</u>				
SPECT Contract	0	1	0	177
SBIR	2	1	216	250
<u>TOTAL CONTRACTS</u>	2	2	216	427
<u>TOTAL DIRB BUDGET</u>	158	159	33,711	36,436

\*Number of grants to be funded FY89 is estimated.

## DIRB ACCOMPLISHMENTS

### CLINICAL DIAGNOSTIC IMAGING RESEARCH TRIALS

The establishment of the National Collaborative Diagnostic Imaging Group is one of the major accomplishments of the Diagnostic Imaging Research Branch. Seven grant awards including an operations control center and a statistical center have been made (RDOG I) in FY87. The objective of this clinical research is for determining the most effective imaging procedures required to stage and monitor carcinoma of the lung and prostate. The research will result in the development of specific algorithms for the appropriate sequential use of state of the art imaging procedures in the diagnostic staging and follow-up of these tumors.

The six funded institutions comprise the membership of the collaborative group. These institutions are evaluating the ability of the newly developed technologies to detect cancers and to determine extent, that is, to stage the disease by employing a single modality or combination of modalities.

Patient accrual in most institutions is ahead of schedule. When sufficient data are accumulated and analyzed, specific decision trees or algorithms for the appropriate sequential use of state-of-the-art imaging procedures in the diagnosis, staging, and follow-up of these specific tumors will be reported.

Five new institutions have been added to the existing collaborative group in FY89. They will study the staging and monitoring of colorectal and pancreas carcinomas. This new group (RDOG II) will be an integral part of the Clinical Diagnostic Imaging Research Trials. They will use the existing operations control center at the American College of Radiology and the Statistical Center at Harvard University.

### RADIOPHARMACEUTICAL DEVELOPMENT FOR SPECT

An RFP, (NCI-CM-57744-26) entitled, "Single Photon Radiopharmaceuticals for Function, Metabolism and Tissue Localization" was issued in FY88. Two proposals were received and reviewed. One will be funded in FY89. Single photon emission computed tomography (SPECT) is a promising technology for non-invasive anatomic and functional diagnosis. The funded contract will conduct research in the designing, synthesizing, labeling and initial testing of radiopharmaceuticals which may be biomedically useful for diagnostic imaging and cancer detection. An aspect of this research will involve the use of monoclonal antibodies as tissue-specific probes for diagnostic imaging.

### DIAPHANOGRAPHY

A four-year clinical trial is nearly completed in which nearly six thousand women were examined for benign and malignant breast disease using a relatively new diagnostic imaging method called diaphanography. The breast is scanned by the transmission of light and near-infrared (IR), radiation from a hand-held probe in contact with the breast. The received radiation is received by a TV camera tube sensitive to light and IR. The optical signal data is filtered, analyzed, and presented as a realistic color-coded image on a TV display. Data are also recorded on optical disks.

All subjects were also given physical examinations and x-ray mammography. The data are not yet completely analyzed. The new method is not intended or expected to be a substitute for mammography as a stand-alone screening tool, but may well have value as an adjunctive



modality along with the physical examination and mammography in decreasing false positives and improving the specificity of breast diagnosis.

## NEW PROJECTS

### A. Five new initiatives have been announced in FY88 and funded in FY89.

1. RFA 87-CA-33, entitled "Development, Evaluation and Biodistribution of Chelate Conjugated Radiolabeled Monoclonal Antibodies Specifically for Diagnostic Imaging", has been announced and applications were reviewed for funding in FY88. This initiative is intended for improvement of existing and development of new chelated monoclonal antibodies which appear to be the most promising for diagnostic purposes. In addition, Phase I Clinical Trials are included in this effort. There exists the possibility of cell-specific diagnosis and the capability of detecting tumors beyond the range of other currently employed imaging modalities. Two applications were funded in FY89.
2. RFA 87-CA-36, entitled "Anatomic and Functional Diagnosis Before, During and Following the Treatment of Neoplasms Employing Imaging Related Technology (MRI/MRS, PET, SPECT, Monoclonal Antibodies) for the Purpose of Planning of Treatment and Monitoring of Tumor Response" was announced and reviewed in FY88. The relative applicability of the various technologies (often used in combination), will be assessed to determine what modality, or what combination of modalities is most suitable, in a given situation for both anatomic and functional diagnosis. Functional information obtainable by imaging and imaging related methods includes the evaluation of various metabolites and determination of such parameters as PH, the tissue redox state and regional tissue perfusion by magnetic resonance spectroscopy. Positron emission tomography (PET) studies also offer opportunities for obtaining similar information. Two applications were funded in FY89.
3. RFA 88-CA-02 to supplement the existing Cooperative Agreement (RDOG) studying lung and prostate cancer was announced. The new awards will be used for staging and monitoring, by imaging methodology, cancer of the colon/rectum and pancreatic tumors. Five new institutions were funded in FY89 and are added to the on-going collaborative group, RDOG.
4. RFA-88-CA-10, "Investigation of Tissue Composition and Function by MRI Using Paramagnetic and/or Superparamagnetic Contrast Agents" was issued in FY88 to solicit grant applications on this subject. The objective of this study is to promote MRI studies employing paramagnetic and superparamagnetic substances in an effort to determine tissue composition, function, localization of tumors, and quantitative measurement of physiological and pathological processes. Eighteen applications were received, and three were funded in FY89.
5. Single photon emission tomography is a promising technology for non-invasive anatomic and functional diagnosis and is much less expensive than PET. The major aim of this RFP is designing, synthesizing, labeling and initial testing of radiopharmaceuticals (radiotracers) which may be biomedically useful as probes of physiologic processes suitable for imaging with the gamma-camera, and single photon emission computed tomography (SPECT). The number of radioisotope combinations now available is limited.

B. Three new initiatives have been concept approved in FY89.

1. National Collaborative Clinical Trials (RDOG III) for Head and Neck and Musculoskeletal Tumors. The objective of this RFA is to diagnose, stage and monitor head and neck and musculoskeletal tumors employing single or multiple technologies and to develop algorithms for the appropriate sequential selection of these diagnostic procedures. Five or more institutions are expected to be funded and added to the existing RDOG.
2. Clinical Diagnostic Studies of AIDS-Affected Organs Using PET and Other Modalities. The objective of this RFA is to stimulate clinical diagnostic imaging research using PET and other modalities to develop new imaging and monitoring approaches for AIDS-affected organs. The brain will be the initial organ site to be investigated. The newly developed methodologies will assist in the determination of patients' clinical status, effectiveness of therapy, and management of patients. Five grants are expected to be funded.
3. Clinical PET Studies of Brain Tumor Metabolism. The aim of this RFA is to advance the use of PET in evaluating essential features of tumor metabolism to improve our knowledge of tumor growth, patient therapy, and patient prognosis and management. Five applications are expected to be funded.

## FY89 Annual Report Summary Diagnostic Imaging Research Branch

The Diagnostic Imaging Research Branch (DIRB), Radiation Research Program (RRP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), supports and administers research leading to the development of radiologic instrumentation and methodology utilizing ionizing and nonionizing radiations to improve the diagnosis of cancer and other diseases. The ultimate goal is non-invasive specific anatomical and functional diagnosis.

Several modalities, both ionizing and non-ionizing, are employed in the field of diagnostic imaging which includes diagnostic radiology and nuclear medicine. In the "Ionizing Section", equipment developments, especially digital radiography, have resulted in improved x-ray technology. Nuclear medicine remains the most active field in the DIRB research effort. In the non-ionizing area of research, such as magnetic resonance imaging/spectroscopy, light scanning and ultrasound, increased activity is evident, especially in MRI/MRS research.

### GRANTS AND CONTRACTS

There are three mechanisms of research support at DIRB; (1) grants, (2) contracts, and (3) collaborative agreements. Grants constitute most of DIRB's portfolio. Traditional grants (R01) leading the way in 1989 with 99, program projects (P01) with 9, cooperative agreements with 12 and others with 28. A new contract was awarded in FY89, "Single Photon Radiopharmaceuticals for Function, Metabolism and Tissue Localization". The estimated budget for this contract is \$177,000. The estimated total expended for grants for FY89 is \$36,009,000 as compared to the 1988 figure of \$33,495,000.

The total number of contracts in DIRB in FY89 is one regular contract and 1 small business contract (SBIR) at a total budget of \$427,000.

### RADIOLOGIC DIAGNOSTIC ONCOLOGIC GROUP (RDOG)

The group is commonly known as the Radiologic Diagnostic Oncology Group (RDOG) and was established in response to an RFA for National Collaborative Diagnostic Imaging Trial Projects in September 1987, and patient accrual convened in November 1987. The group's objective is the timely evaluation of current and emerging imaging modalities in the management of patients with cancer. The findings of each study should lead to improved patient management and considerable cost savings resulting from the elimination of inappropriate or unnecessary diagnostic studies. Furthermore, the development of clinical trial groups allows for the rapid accrual of patients into a study within a short period of time. This assures rapid evaluation of imaging modalities for imaging procedures in diagnostic staging in cancer patients and timely follow-up of their tumors.

The clinical trials group is currently evaluating carcinomas of the lung and prostate. Semi-annual reports indicate satisfactory progress, especially patient accrual. Patients accrual with prostate carcinoma is ahead of schedule and that of the lung is slightly lower. A second RFA for clinical trials involving carcinoma of the pancreas and colorectal carcinoma was announced, and applications were received and are being reviewed.

The clinical diagnostic imaging trials of colorectal and pancreatic cancers (RDOG II) have been recently added to the ongoing collaborative group. Five new institutions were added, namely, Johns Hopkins, Washington University (St. Louis), University of Washington, Seattle, University of Michigan and New York University, NY. The new institutions will join the six

institutions (RDOG I) currently participating in the clinical trials. Institutions included in RDOG I are the Cleveland Clinic, Johns Hopkins University Hospital, Memorial Sloan-Kettering Cancer Center, Thomas Jefferson University Hospital, University of California at San Francisco and the University of Michigan at Ann Arbor. An operations control center managed by the American College of Radiology and a Statistical center operated by Harvard University constitute the remaining members of the RDOG. It is anticipated that five or more new institutions to study head and neck and musculoskeletal carcinomas will be added to the RDOG in FY90.

The clinical trials will contribute significantly to the development of a comprehensive comparative teaching file by site. This should prove to be an excellent resource for training radiologists in the selection of the optimal imaging modality and for performing additional studies on the impact of external factors on radiology interpretations. Moreover, the clinical trials will stimulate spin-off projects addressing some of the questions that are not within the scope of the grant. Potential research projects will involve detailed studies of MR tissue characteristics, predictive factors for sites of disease involvement, impact of workup bias on test performance, and many other important areas in clinical diagnostic radiology.

#### DIGITAL RADIOGRAPHY AND OTHER TECHNOLOGY DEVELOPMENTS

Program took a leading role in supporting research in the development and utilization of electronic and other imaging technology. Research in areas of digital electronic communication technology, picture archiving and communication systems (PACS), image acquisition and sorting, and image enhancement has been actively pursued by DIRB.

The unique expertise of a research team at the University of Pittsburgh, in high resolution storage phosphor imaging, fast digital acquisition, and PACS brought several advances in this vital area of imaging research. They were able to assemble and evaluate a multi-port digital radiography system based on two-dimensional solid-state arrays which are fiberoptically coupled to either a fluorescent screen or a scintillating fiberoptic faceplate. They further assembled a digital, real-time, multi-port x-ray camera fiberoptically coupled to a fluorescent screen. The camera is capable of producing a high quality, high-resolution x-ray image. This perhaps is the first and only operational system of its kind.

At the University of Chicago, grantees are developing computerized schemes for the detection of lesions such as clustered microcalcifications in mammograms and lung nodules in chest radiographs, in order to enhance images to assist radiologists' diagnoses. The newly developed computer detection system is based on a difference-image technique in which a signal-suppressed image is subtracted from a signal-enhanced image to remove the structured background in radiographs. Feature-extraction techniques are then applied to the difference image in order to isolate lesions. The true positive detection rates for clustered microcalcifications and lung nodules are currently 90% and 70%, respectively. Such a device will significantly improve mass-screening procedures in lesion detection in mammography, lung nodules or other related uses.

At the University of North Carolina, the primary objective of their research program is to develop improved means of medical image processing utilizing data obtained from tomography (SPECT) imaging systems as well as radiation therapy port films. Efforts are being made: a) to produce better grey-scale 2D images by utilizing regionally adaptive contrast enhancement techniques and targeting context-sensitive-human vision; b) to develop dynamic presentation of 3D images both for anatomical and functional information; and c) to define anatomic objects for fast 2D/3D object definition. This team is in the process of developing a state-of-the-art imaging facility far ahead of many other institutions. A visit to such a facility is the only way to

appreciate the advanced state of technology and advanced level they have reached in image transmission and display in both two and three dimensions.

A pioneering effort at UCLA for handling, examining, and sorting the vast number of radiological images is now being generated on film and being sorted electronically by means of digital radiography. This project has resulted in a new image viewing console which has six television monitor displays on which images can be recalled, selected, magnified, examined, and compared in a rapid and flexible mode of operation. The monitors presently display 1000 X 1000 pixels in each image with a spatial resolution on the tube displays exceeding conventional radiographic films (except for mammography) and 2000 X 2000 pixel displays will be installed in the coming year. Laser-printed hard copy is available. Although primary diagnoses are made by film, all subsequent interpretation and storage has been converted to digital (or photo-electronic) operation in the Pediatric Radiology Department.

### MONOCLONAL ANTIBODIES

The use of monoclonal antibodies in diagnostic imaging is of great interest to our program for some time. An RFA was issued two years ago as a result of a workshop. The number of grants supported in this field is increasing and important publications have resulted from this effort. Grantees have investigated several of the most promising monoclonal antibodies (MoAbs) reactive with human colorectal cancer (35,115, B72.3 and 17-1A) for heterogeneity in binding between and within colorectal tumor masses from human patients, and in biodistribution and therapy studies in athymic nude mice bearing human colorectal tumor xenografts. These studies were designed to look at the heterogeneity of binding and localization of the different MoAbs. The results confirmed the hypothesis that there would be heterogeneity with respect to absolute tumor uptake. They found that human-mouse chimeric variants of murine MoAb 17-1A showed similar binding, tumor uptake and therapeutic efficacy in colon tumor xenografts as the murine MoAb. These results suggest that chimeric 17-1A MoAbs might be promising in clinical localization and radioimmunotherapy studies.

In recent investigations at the University of Michigan, the use of radiolabeled monoclonal antibodies to detect for breast carcinoma has been explored. Nine patients have been studied by the subcutaneous administration of the I-131 labeled B72.3 monoclonal antibody, and eleven patients have received the <sup>111</sup>In labeled 103-D-2 monoclonal antibody intravenously. Satisfactory tumor imaging has been achieved with both agents in many instances, but neither appears sufficiently sensitive to replace axillary nodal dissection as a method of non-invasively evaluating regional draining lymph node histology. Additional studies are under way to improve this method. In another study using monoclonal antibodies in the diagnosis and treatment of ovarian cancer, they found that antibody fragments appearing to have pharmacokinetic advantages over intact antibodies. They also found that the diaphragm is the major route of delivery and that delivery can be enhanced by diaphragmatic blockade. These observations support a rationale for evaluation of the intra-peritoneal delivery of monoclonal antibody fragments for tumor imaging and treatment. These preliminary results indicate the bright future of the use of monoclonal antibodies in both image diagnosis and cancer therapy.

Quantitative whole-body autoradiography (WBAR) provides a high-resolution imaging system for analysis of specific sites of tissue localization of anti-tumor radioantibodies. WBAR data indicated that improved imaging of xenografted human colon carcinoma at early time points is made possible by use of the F(ab')<sub>2</sub> and Fab' fragments of anti-carcinoembryonic antigen monoclonal antibodies (MoAbs). WBAR images also revealed that use of second antibody in a xenograft system leads to rapid clearance of radiolabeled primary antibody and thus enhances tumor imaging by radioantibodies. A recent study shows that the application of WBAR in a

xenograft tumor model, injected with  $^{90}\text{Y}$  labeled MoAb, revealed bone to be a major site of  $^{90}\text{Y}$  uptake, explaining the myelosuppression noted in therapy trials with this nuclide.

## NUCLEAR MEDICINE

Research in nuclear medicine comprises a major portion of traditional research grants (R01) in DIRB. Development of new radiolabeled compounds, their biodistribution, toxicity, use in various modalities, i.e., PET and SPECT, and chelate conjugated antibody research are just a few examples to illustrate the wide range of this program. Program project grants deal with various aspects of nuclear medical research. One deals with the development and evaluation of promising compounds which when radiolabeled, have the potential to scintigraphically diagnose and at higher doses treat cancer. A second program project continues to explore the improvement of image information by the administration of a novel contrast agent; improvement of the qualitative and quantitative aspects of imagery by SPECT and utilization of pharmacoangiography to improve information content and understanding of functions of imaged organs. An important development by another program project is to design and develop a scintillation probe for intraoperative tumor detection. By using two detectors, the newly constructed probe effectively discriminates against background radiation that might otherwise be mistaken for tumor, improving detection of small metastases when compared to external imaging or other surgical probes. It is expected that a commercial probe will be constructed based on this prototype and used routinely in surgery.

Technetium-99m HM-PAO is an efficacious cerebral perfusion agent which is currently being marketed in Europe and the US. The inherent instability of this agent underlies the in vivo mechanism by which it is trapped in the brain, but it also limits the clinical utility of Tc-99m HM-PAO since this agent must be administered within 30 minutes of its formulation. Studies at the University of Cincinnati on the mechanism of action of Tc-99m HM-PAO have led to the development of Tc-99m CB-PAO, which is just as effective as HM-PAO in SPECT imaging, but which can be used up to six hours after formulation. This new CB-PAO agent should allow realization of the true clinical potential of PAO-based cerebral perfusion imaging agents.

At the University of Washington in Seattle, studies of the biochemistry of thymidine using PET continue to show that de-iodination of I-125-IUDR was occurring in vivo and that there is little local thymidine reutilization. While the degradation pathway appears active in slowly growing tissues, it appears to be less of a concern in rapidly growing tissues and tumors, where these studies found between 75 and 95% of retained thymidine is incorporated into DNA. This information along with previous work on the effects of blood flow and endogenous synthesis are being used to produce models of thymidine metabolism for PET.

Significant progress was made at Hunter College in their study of their new porphyrins (N-benzyl-tetrakis (4-carboxyphenyl) porphine). They found that it can rapidly bind radioactive metals such as  $^{67}\text{Cu}$ . This metal complex localizes selectively in stressed lymph nodes, providing a possible diagnostic tool. They have also found that porphyrins can be bound as their active metal complexes to antibodies without substantial loss in immunoreactivity. In addition, one of these porphyrins (N-benzyl-5-carboxyphenyl-10, 15, 20 - 4 sulfonatophenyl porphine) binds  $^{67}\text{Cu}$  rapidly when it is linked to the antigenic fragment of the human acetylcholine receptor. The complex is immunoreactive, providing a possible therapy for myasthenia gravis.

The use of single photon emission computed tomography (SPECT) as a clinical modality in diagnostic imaging is well established. Over two thousand SPECT systems are installed worldwide. In addition to diagnostic application, SPECT also has the potential to provide quantitative information on the effectiveness of cancer therapy, e.g, change in size of primary

or metastatic lesions following therapy. The Diagnostic Imaging Program is supporting research leading to the construction of new and improved SPECT systems, improvement and development of SPECT, and development of radiolabeled compounds, especially using Tc-99m for use in SPECT.

Significant research accomplishments at Duke University have been made in the areas of new and improved collimator geometries for SPECT as well as in the development of new statistically based reconstruction algorithms. In the first area further refinements have been made in the use of cone beam collimation for SPECT. This method to improve detection was first proposed with prior research funded by the grant. The significance of cone beam collimation is that this geometry provides a two- or three-fold increase in the number of detected events, without degrading the reconstructed spatial resolution. Thus, image noise is reduced, and diagnostic utility is enhanced. Development of similar instruments at the University of Michigan were able to experimentally characterize the performance of their (SPRINT II) capable of imaging human brains and young pediatric patients with a resolution of 8-9 mm at a sensitivity four times greater than the conventional Anger camera. Not only will this improve patient throughput and improve image quality, but it opens the door to performing dynamic tomography.

#### MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY (MRI/MRS)

Basic and developmental research studies of many kinds have continued to make rapid and revolutionary advances in improving the equipment, materials, and techniques for clinical diagnostic imaging, noninvasive tissue characterization, and studies of normal and pathologic tissues by MRI and MRS.

In the improvement of instrumentation and techniques highly successful developments have been realized in the design of surface coils and other special electronic coils for imaging all parts of the body, for localization of tissues, and for spectroscopic determinations. Optimal magnetic shielding designs have been developed as well as phantoms for quality control measurements.

Major interest has been centered on the development and evaluation of new MRI contrast agents, such as paramagnetic iron and gadolinium complexes, to be used as hepatobiliary contrast agents. Stable liposomes of manganese cations and nitroxide free radicals alter the NMR relaxation rates of liver tissue and intracellular water and enhance MR image contrast. Brain tumors and edema can be differentiated using gadolinium (DTPA); magnetic fluorine nuclei are being used to study 5-Fluorouracil chemotherapy and to detect tumor sensitivity or resistance; and special immunodirective agents, including monoclonal antibodies, can be incorporated with contrast agents to improve the specific imaging of tumors.

Basic research studies continue to measure the magnetic resonance relaxation properties (T1 and T2) of normal and pathological tissues as well as differences in the electrochemical characteristics of physiological water in normal and cancer cells, the transformation from preneoplastic to metastatic hepatocellular carcinomas, and the metastatic potential of prostate cancers.

New improvements developed by physicists and electrical engineers in applied NMR techniques have permitted high speed, real-time dynamic imaging of the beating human heart and of vascular flow patterns. High magnetic field spectral imaging and chemical shift imaging techniques now can display and measure intracellular and extracellular sodium in the assessment of cancers, stroke, and trauma and can display lesions not seen without these techniques. It is now possible to use MRI to assess both perfusion of organs and diffusion within cells, to image only the aerated portions of lungs, and to study the actions of macrophages in lung

tissues. New developments in magnetic resonance microscopy promise to provide magnified, noninvasive imaging of tissues at a spatial resolution of ten microns in the near future.

Clinical evaluations conducted over the past few years have shown that MRI and CT are approximately equally useful in detecting and visualizing tumors in the brain, although the multiplane viewing capability of MRI offers additional detailed flexibility in imaging. MRI is superior in viewing demyelinating disease, such as the lesions of multiple sclerosis. It is especially good in visualizing in very fine detail the disorders of the cervical spine, so that a majority of myelographic examinations (but not all) formerly used for the spine may be obviated. MRI is excellent in diagnostic imaging of joints and is particularly valued by surgeons replacing hip joints for presurgical three-dimensional planning of sizes and fits. Pelvic regions of male and female are well seen by MRI as well as by CT, but CT is favored in abdominal organs for its speed, eliminating motion artifacts that arise from respiratory and peristaltic motions.

Other specific applications where MRI has been found especially useful are in the demonstration of smaller acoustic neuromas and pituitary tumors and in the detection of a variety of intracranial hemorrhagic conditions after 48 hours and of early changes in aseptic necrosis of bone. CT remains the method of choice for staging bronchogenic carcinoma and in evaluating small lesions of the pancreas and spleen and metastases in the lymph nodes.

#### ULTRASOUND IMAGING AND CHARACTERIZATION

Several projects to improve the technology of ultrasound imaging systems have continued. Some of these involve computer ultrasound transmission tomography systems designed for regions of the body that are composed of non-bony soft tissues, such as the breast or abdominal organs. Another system uses a combination of reflection and transmission tomography and has also demonstrated the beginning of three-dimensional real time visualization of a rotating object, such as the hand. Another system is also aimed at 3-D real time ultrasound images based on the high speed parallel processing of received ultrasound data.

Developments continue on methods for improving ultrasound signal detection by reduction of speckle, by enhancing contrast, by providing computerized image processing to correct for or eliminate sonographic artifacts, and by the use of Doppler ultrasound to detect tumors. Doppler techniques applied to tumor detection in the vascular system in and around tumors has been considerably enhanced by the success in use of two-color Doppler to differentiate forward blood flow from retrograde flow or arterial from venous flow.

Progress has been made in the noninvasive characterization of tissues by ultrasound, and in differentiating cyst from solid and benign from malignant conditions in breast disease. Greater emphasis is being placed on evaluating more than one ultrasonic property of tissues at a time, such as attenuation, speed of sound, and reflectivity, instead of only one parameter. This permits parametric display and multivariate analysis of the signal data that make up the image, enabling differential color encoding of malignant tissues in ultrasound images and assisting the physician in making differential diagnostic correlations.

New statistical approaches to tissue characterization are finding practical application in detecting and assessing tumors of the liver and other organs by ultrasound. The new mathematical technique of fractal geometry is also being used to differentiate malignant from benign liver tissue based on ultrasound data.

Fundamental to all advances in ultrasound imaging and tissue characterization is the increase in knowledge gained from continuing investigations by many grantees studying non-linear



propagation and scattering of ultrasound energy in tissues by direct studies in tissues and in phantom models.

Several expert investigators in the longterm programs studying potential bioeffects of ultrasound energy exposure to tissues at levels in the diagnostic range provide new knowledge in this area and the basis for being assured that no significant biological hazard is present in conventional diagnostic ultrasound imaging practices. The present emphasis is on achieving a complete physical understanding of the nature of transient and stable cavitation phenomena.

#### SMALL BUSINESS INNOVATIVE RESEARCH GRANTS AND CONTRACTS

There have been 21 Phase I SBIR grants, 9 Phase II SBIR grants, and 2 Phase II SBIR contracts active during FY88. The grants cover areas of DIRB program interest which include research and development in nuclear magnetic resonance, ultrasound, x-ray instrumentation and digital radiography, monoclonal antibodies, electronic and software development, microdetectors, and controllers and many other highly useful diagnostic imaging devices.

One SBIR grantee has developed the fastest MRI technique, which produces images in less than 60 milliseconds at a rate of 16 images per second, permitting the real time cardiac imaging referred to above. Other SBIR grants are devoted to development of tumor-specific MRI contrast agents and special coils, and one has developed a method for screening MRI patients for the possible presence of implanted magnetic prostheses, similar to an airline baggage scanner.

Another SBIR contractor has made progress in the development of sophisticated high frequency, high power amplifiers, switches, and circuitry for commercial use in the magnetic resonance imaging systems (MRI) of larger commercial manufacturers. Another grantee has succeeded in making ferrite-based MRI contrast agents which are immunodirectable and can be selectively bound to red blood cells.

A computed tomography breast scanner based on reflected ultrasound has been developed with excellent spatial resolution in all directions. It is able to image small ( $< 0.5$  mm) ultrasound reflecting particles similar to microcalcifications. The same grantee is also developing an "expert radiology assistant" system to facilitate the task of the x-ray mammographer in detecting and classifying lesions. Another development has produced an x-ray sensor for use in quality control and dose reduction in clinical mammography. An interesting linear holographic ultrasound scanner utilizing a band of discrete frequencies has demonstrated increased depth resolution and the possibility of producing a B-scan ultrasound image of an object without mechanical or electronic scanning. A microwave radiometry scanning system to detect breast cancer by detection of thermal anomalies is under development. Another SBIR grantee has developed a catheter-tipped ultrasonic transducer which can be used to direct laser surgery procedures delivered through fiberoptic catheters.

#### WORKSHOPS

The most important goal of the DIRB workshops is to determine the future directions of diagnostic imaging research and to develop scientific initiatives in the areas identified by the leaders in diagnostic imaging and related sciences as most important to pursue. Two workshops were held in FY89.

## "Directions in Research in Diagnostic Imaging" October 12-14, 1988

A two-day workshop sponsored by DIRB was held in Winston-Salem, NC in collaboration with the Department of Radiology at Bowman Gray School of Medicine. The workshop was designed to update the research plan for diagnostic imaging published in the May/June 1984 issue of Investigative Radiology. The proceedings of this workshop will be published in the same journal and will assist DIRB in developing new initiatives for future RFAs, RFPs, Collaborative Clinical Trials and Program Announcements.

## "Monitoring of Tumor Response to Therapy by MRS/MRI" September, 1989

This workshop is planned for September, 1989. Magnetic resonance spectroscopy (MRS) aided by MRI is demonstrating increasing promise in evaluating and following changes in tumor metabolism following radiation therapy, chemotherapy and hyperthermia, e.g., by variations in intensity of phosphorus-31 spectral peaks. Now it is also possible to use this tool to assess the effects of hormonotherapy and immunotherapy. MRI is used to orient and localize the spectroscopy measurements and also to determine differences in size, shape and distribution of tumors following treatment. High magnetic fields (2.0 Tesla or above) are preferred to achieve sensitivity. Various combinations of therapeutic modalities as in present day oncologic practice will also be considered.

## DIAGNOSTIC RADIOLOGY COORDINATING COMMITTEE (DRCC)

This committee (DRCC) was created 1989 to replace the NIH Inter-Institute Diagnostic Imaging Group (IDIG). IDIG was established in 1986 to promote collaboration among NIH institutes and facilitate the dissemination of information concerning diagnostic radiology and imaging research among the institutes having programs in these areas.

In order to have a focal point to oversee the coordination of NIH-wide imaging activities, the Director of NIH designated the National Cancer Institute to be the lead Institute responsible for the development of the new committee (DRCC). The Radiation Research Program, Division of Cancer Treatment has been identified by the NCI to direct this committee.

The DRCC has representatives from each of the Institutes and other NIH component groups, both intramural and extramural, which have significant interests or programs in diagnostic imaging. The possibility of having scientific advisors from non-governmental clinical, scientific, and computer science organizations has been explored and will be considered further. Such a group could provide valuable expertise about the technological and clinical aspects of all of the diagnostic imaging modalities and their research potential. There is general agreement that a large amount of information is available concerning NIH resources and activities (both intramural and extramural) that can be shared and disseminated among the various Institutes. The Committee is responsible for the coordination of diagnostic imaging research at NIH, developing a NIH-wide long-range research plan for diagnostic radiology, and reporting on a regular basis to the Director, NIH.

## FUTURE DIRECTIONS (DIRB)

1. Multinstitutional Imaging Trials will need to be expanded beyond their current activity. As research in this area expands, both the disease sites to be studied and the number of institutions participating will be increased. Additional funding is thus necessary. New advances in technology will be clinically tested. Head and neck, musculoskeletal, breast, and liver are sites being considered for addition to the RDOG.

2. For decades breast cancer has been the leading cause of death from cancer in women and has shown an apparent recent increase in incidence. Although x-ray mammography is the primary tool for detection, clinical trials by Dempsey et al ("Ultrasound Quarterly," Issue No. 1, Fall 1987) have shown that the use of x-rays plus ultrasound imaging improves the rate of early detection of malignant lesions. However, less than 15% of American women even use breast self-examination or avail themselves of baseline mammography. Several non-ionizing methods of imaging coupled with tissue characterization should be developed further (see next item). MRI is useful for tissue characterization as well as diagnosis, but it cannot be used for screening because of cost and slowness of patient imaging. Ultrasound imaging and Doppler flow, thermal measurement, transillumination, and photon time delay spectroscopy and imaging all have further unexploited potential for non-invasive characterization of breast abnormalities.
3. A DIRB workshop on "Ultrasound Tissue Characterization" indicated that the next stage of development for ultrasound imaging should be the use of multivariate analysis and image processing techniques for improved tissue characterization and the extension of ultrasound systems to higher frequencies for the special uses in the characterization of surface tumors and skin lesions and as endocavitary and intraoperative probes.
4. MRS has important potential for further study and staging of tumors and for monitoring the effects of radiation therapy, hyperthermia, chemotherapy, and immunotherapy. A workshop on the uses of MRI/MRS to monitor tumor response to therapy is being planned for the fall of 1989.
5. New progress in the development of picture archiving and communication systems (PACS) have brought about the need for new software management tools from the field of medical informatics, a new and growing science concerned with the development of decision support tools, data management and physician workstation environments that increase the efficiency and personal productivity of the diagnostic radiologist. New initiatives are expected that will stimulate research and development of knowledge-based systems directed at diagnostic imaging applications. These systems, coupled with PACS networks, will eliminate the need for the patient's traditional x-ray film file which is now tracked by each department care facility. These computer-based systems will improve efficiency, quality control and bring new capabilities to the physician.
6. The "Clinical Applications of Positron Emission Tomography" was explored in a DIRB workshop held September 14-16, 1988. Advances in the use of this diagnostic modality are bringing to reality the ability to differentiate normal tissue from tumor tissue.



## B. RADIOTHERAPY DEVELOPMENT BRANCH

The Radiotherapy Development Branch (RDB) continues to develop and administer a large program of basic science and clinical research activities related to cancer treatment. The disciplines represented are radiation oncology, radiobiology, radiation chemistry and radiation physics. Research efforts range from investigation of the basic physics and biological effects of radiation to controlled clinical trials for a variety of neoplastic diseases and therapeutic modalities.

Major areas of funding are in particle radiation therapy, radiosensitizers, photodynamic therapy, systemic radiation therapy (SRT), radiation physics, and hyperthermia and its associated biology. An area of increasing interest and importance is boron neutron capture therapy (BNCT).

The following is the RDB budget for FY88 and the estimated budget for FY89.

### FY88 and FY89 RDB Budget

	FY88	FY89	\$(thousands)	
			FY88	FY89
GRANTS				
Traditional (R01)	176	159	29,936	28,099
Program Projects (P01)	13	14	13,906	18,563
Conference and New Investigator (R13 & 23)	4	1	80	5
First Awards	11	12	947	1,048
Merit Awards	7	7	1,878	1,859
Cooperative Agreement (U01)	1	1	1,319	1,383
Other	0	0	0	0
SBIR	8	9	1,183	1,583
TOTAL GRANTS	220	203	49,249	52,540
CONTRACTS				
Regular	12	14	5,322	4,967
SBIR	6	4	1,244	200
TOTAL CONTRACTS	18	18	6,566	5,167
TOTAL RDB BUDGET	238	221	55,815	57,707

\*Not all FY89 SBIR awards have as yet been determined.

### FY89 Annual Report Summary Radiotherapy Development Branch

The Radiotherapy Development Branch (RDB) administers a large program of basic, developmental, and clinical research related to cancer treatment utilizing ionizing and nonionizing radiations. Radiation research encompasses a range of scientific disciplines including biology, chemistry, physics and clinical oncology as well as the specialized treatment modalities of photodynamic therapy and hyperthermia. More recently, the role of computer-

based tools for the diagnosis, therapy selection and radiotherapy treatment planning processes have received increased emphasis in the Program. Research efforts range from the investigation of basic mechanisms at the atomic and cellular levels to controlled clinical trials for a multitude of diseases using single or multimodality treatment schemes.

Basic research supported by RDB has generated leads for promising new treatment modalities that are currently being tested in clinical trials. Major areas of funded research include particle radiotherapy, hyperthermia, and general radiobiology. Substantial support is also provided for the development of radiomodifiers, tagged antibody therapy, boron neutron capture therapy, photodynamic therapy, and radiation physics. Radiation modifiers are being explored as protective agents to reduce normal tissue morbidity, and as sensitizers to enhance the effects of radiation on tumors. Advanced treatment planning tools continue to be developed through a series of collaborative working groups that are bringing three-dimensional computer graphics and decision-support tools to the treatment planning process.

## PARTICLE RADIOTHERAPY

Radiotherapy with either charged or uncharged particles continues to receive a significant portion of the RDB budget. Neutron therapy Phase III trials compare fast neutrons against best conventional photon therapy for head and neck cancers, prostate and lung tumors, as well as tumors of radioresistant histologies, such as sarcomas of the soft tissue and bone and melanoma. Charged particle therapy with both protons and heavy ions are now successfully treating a variety of tumors in the lung, prostate, eye as well as lesions adjacent to the spinal cord that cannot be treated with any other therapy. Results of treatment of tumors such as uveal melanomas and chordomas and low grade chondrosarcomas of the base of the skull and the cervical spine show a major improvement over conventional x-ray treatment methods. Because of the sparing of adjacent normal tissue with protons or heavy ions, higher tumorcidal doses can be delivered to these lesions which are not attainable with conventional therapies.

Limited Phase II trials with protons are planned or underway for three sites: carcinoma of the nasopharynx, Pancoast carcinoma of the lung with extension into the vertebral body, and sarcomas of the sacrum which are incompletely excised or inoperable. The expectation is that these experiences will be the basis for Phase III trials of protons vs. photon beam therapy. A Phase III trial is also planned to compare fractionated radiation vs. single dose for arteriovenous malformations of the brain. A Phase III trial currently underway which compares protons versus photons for a prostate treatment shows that a proton boost to 74 Gy is tolerated as well as a photon boost to 68 Gy. The increased dose to the prostate is possible with protons because of normal tissue sparing.

At Lawrence Berkeley Laboratory in California, initial results with neon ions in Phase I/II studies were sufficiently promising to plan prospective trials for a number of sites. Randomized Phase III studies have been established for the treatment of unresectable lung and locally advanced prostate tumors. A Phase II study will soon open for glioblastoma comparing neon ions with the well established photon database of the University of California (San Francisco) Brain Tumor Research Center. A randomized Phase III neon vs. helium ion study is being developed for base of skull and thoracolumbar paraspinal tumors, sarcomas of soft tissue and bone, and locally advanced lesions of unusual histology such as renal sarcoma, thyroid cancer and melanoma.

Phase III neutron therapy clinical trials continue, following completion of the Phase I/Phase II studies which determined the maximum tolerated neutron doses for the head and neck, thorax, abdomen, pelvis and extremities. The Phase III trials are comparing fast neutron radiation therapy versus the best conventional photon treatment for squamous cell carcinomas of the head

and neck, non-small-cell lung cancers, prostate cancer and tumors of radioresistant histotypes including sarcomas and melanomas. Two other Phase III studies, rectal and cervix cancer, were terminated because of inadequate patient accrual. Accrual to the head and neck studies is progressing steadily and is projected to be complete by 1993. The lung and prostate trials will reach completion in the next 1-3 years. Approximately 1700 patients have been treated with fast neutrons on new state-of-the-art equipment since the NCI initiative was launched in 1979 to develop and test hospital-based fast neutron treatments against best conventional therapy.

## HYPERTHERMIA

The large number of grant applications and contract proposals representing all aspects of pre-clinical and clinical studies in hyperthermia attest to the strong interest by the research community in this field. Two major directions in the area of hyperthermia are the development of appropriate devices for the optimum application of hyperthermia to a tumor mass and the elucidation of the molecular or biochemical mechanisms(s) of hyperthermia-induced cytotoxicity and radiosensitization. Studies have directed attention to the mechanisms of heat damage and the factors which modify this effect. Attempts are underway to model the temperature distribution of tumors and to correlate the temperatures achieved with clinical results. Efforts to develop new and more effective hyperthermia applicators and devices are in progress.

A wide variety of hyperthermia effects of hyperthermia have been observed in various pre-clinical studies. Using a temperature sensitive mutant, investigators at the University of California, San Francisco noted that if thermal damage can be repaired very rapidly, there will not be any heat resistance or thermal tolerance observed. This finding may be important in overcoming the resistance of tumors to combined modality treatment (i.e. radiotherapy or chemotherapy with hyperthermia). These same investigators are also studying the mechanism of increased chromosomal aberrations produced when local anesthetics such as procaine hydrochloride are used in conjunction with hyperthermia. Using a new technique called orthogonal field alternating gel electrophoresis to measure damage in large DNA molecules, these investigators are studying the rearrangement of genes in the chromosomes after the cells have been treated with heat and/or radiation. This approach should help determine how these two agents kill cells at the DNA level, but may also shed light on the molecular basis of resistance that is occasionally observed with the use of radiation and drugs in the treatment of cancer.

At Thomas Jefferson University Hospital, the effectiveness of local anesthetics as heat sensitizers was shown to be dramatically influenced by external pH. Caffeine was shown to be a heat sensitizer and its sensitization correlated directly with induced increases of cellular free calcium levels. The calcium channel blockers verapamil and diltiazem were shown to be heat sensitizers and they act synergistically with procaine + heat. Heat induced alterations in the ultrastructure of both the nuclear matrix and nucleoli of mammalian cells were observed indicating functional change in the nuclear matrix. These investigators appear to have developed a system for monitoring repair of heat-induced Potentially Lethal Damage (PLDR). This procedure will have far-reaching applications in the study of mechanisms of heat cytotoxicity by allowing for comparisons to be made between kinetics of repair of specific heat-induced lesions with the recovery kinetics for survival.

The metabolic environment of tumors, in particular the extracellular pH is being investigated. Generally, the extracellular tumor pH is somewhat more acidic than that of normal tissue. Using *in vitro* models, investigators at Case Western Reserve have found that radiation and heat response of CHO (Chinese hamster ovary) and A549 (human lung carcinoma) cells can be enhanced, in a pH-dependent manner, by treatment of cells with certain ionophores and

metabolic inhibitors. For example, the K<sup>+</sup>/H<sup>+</sup> ionophore nigericin inhibits recovery of cells from potentially lethal radiation damage (PLDR) when pHe is 6.5 to 6.8, but has no effect of PLDR when pHe is 7.0 to 7.3. At pHe 6.4 to 6.2 nigericin interacts synergistically with radiation to reduce survival below that of untreated cells. Presently they are studying the underlying biochemical mechanisms of the pHe-dependent effects they have observed so these results can be used in the development of clinically relevant agents.

At the University of Maryland, it was shown that cells in SCK tumors were sensitive to heat and that the sensitivity remained in cultures when the environment was acidic or hypoxic. When the environment was corrected to a neutral pH or aerobic condition, the heat sensitivity changed to a resistant state. This process was reversed by depleting glucose or by lowering the pH of the culture conditions and also by unidentified factors. This suggests that tumor heat sensitivity is modified by previous treatments which are vasoactively alter the tumor microenvironment.

In a study conducted at the Medical College of Virginia, the enhanced rate of generating the reactive oxygen species in tumor tissues is an important mechanism in damaging the endothelial cells of the microvasculature structure. It was observed that at hyperthermic conditions the reaction between xanthine oxidase (known to associate with endothelial cells) and purine (high concentration in tumor) produce toxic oxygen species at higher rate than at normal physiological temperature. The high rate of toxic oxygen production corresponds to greater cytotoxicity in fibroblasts and endothelial cells. The actual toxicity from this reaction was shown to be caused by hydrogen peroxide which can be eliminated by adding catalase or erythrocytes to the experimental system.

Investigators at the Ellis Fischel State Cancer Center have shown that hyperthermic perfusion of rat liver results in lipid peroxidation. A source of oxidative stress was shown to result from the conversion of xanthine oxidase to the oxidase form. This conversion resulted in loss of glutathione from the perfused liver, mainly as oxidized glutathione. Allopurinol protected the liver from the toxic effects of hyperthermic perfusion. These investigators concluded that hepatic dysfunction induced by hyperthermia (42° to 43°) is probably caused by oxidative stress with subsequent lipid oxidation and that allopurinol inhibits the formation of oxygen free radicals and possibly lipid peroxidation.

Numerous pre-clinical studies have been devoted to the thermotolerance and the expression of heat shock proteins associated with hyperthermia. At Washington University, it was noted that exposure to elevated temperatures induce a translocation of hsp 70 from the cytoplasm to the nucleus. This process was found to have similar kinetics in cells displaying various thermal responses as judged by clonogenic survival, including normal, transiently thermotolerant and permanently heat resistant Chinese hamster fibroblasts. This parameter, therefore, cannot be used to distinguish such cells from one another. The return of hsp 70 from the nucleus to the cytoplasm after an initial heat-shock was also examined in the three different cell types. It was found that in cells which had become transiently thermotolerant after a brief exposure to heat or sodium arsenite, and in cells which are permanently heat resistant, the removal of hsp 70 from the nucleus was more rapid than that observed in normal cells after a given heat challenge. Further experiments demonstrated that cells acquire the capacity for the rapid return of hsp 70 from the nucleus to the cytoplasm as clonogenic thermotolerance develops and that this capacity decays with kinetics similar to that observed for the decay of clonogenic thermotolerance. The rate of removal of hsp 70 from the nucleus after a heat challenge was found to correlate with altered heat sensitivity. The thermoresistant state, whether transient, as in the case of thermotolerance, or permanent, as in the case of heat resistant variants, is associated with a more rapid removal of hsp 70 from the nucleus after a heat challenge. These results could have practical application by generating a test for thermotolerance and intrinsic heat resistance in the clinical application of hyperthermia.



Investigators at the University of Iowa found that the HL-60 leukemia cell line synthesized multiple hsp including the highly conserved hsp 70 family (hsp 69/72 doublet.) Cellular differentiation and membrane lipid structural modification had no effect on expression of hsp, however, there was selective failure to express the hsp 69/72 when the heat was delivered at a slow controlled rate.

A glycoprotein (GP50) has been identified by investigators at the University of Utah who showed that GP50 synthesis is increased during thermotolerance development in a variety of mammalian cell lines and that it correlates with thermotolerance expression in a series of thermotolerance-deficient mutants. They characterized heat shock protein and GP50 synthesis under conditions of stepdown heating and showed that the enzymes involved in O-linked, but not N-linked glycosylation, are also significantly increased prior to and during thermotolerance development. In separate experiments, they showed that microinjection of poly(A)<sup>+</sup>RNA, isolated from thermotolerant cells conferred thermotolerance to recipient cells. This finding clears the way for the eventual identification of specific genes involved in the expression of thermotolerance and will be used to determine the relative capacity of heat shock proteins vs. glycoproteins, such as GP50, in mediating cellular heat resistance. Understanding the biochemical and genetic basis of thermotolerance should lead not only to improved applications of hyperthermia in cancer therapy, but also applications in other areas of stress physiology, e.g., agriculture, with the eventual production of more heat-resistant crops and livestock.

Investigators at East Carolina University have used a cytofluorometric technique to demonstrate that intracellular hydrogen peroxide production is increased in the entire cell population and not a selected subset following heat shock or treatments known to induce thermotolerance. Further, thermotolerant cell populations have lowered basal and heat induced hydrogen peroxide production. They also found that induction of thermotolerance induces catalase activity.

It has been stated that the cytotoxicity of some chemotherapeutic agents is enhanced at elevated temperatures. Several studies have examined this relationship. At the Massachusetts General Hospital, investigators treated single cell suspensions prepared from C3Hf/Sed mouse fibrosarcoma with chemotherapeutic agent at various temperatures *in vitro* and compared the activation energy for these agents. Cell survivals were obtained by the lung colony assay method and survival curves were drawn as a function of treatment time. The reciprocal of the slope ( $D_0$ ) was plotted against the reciprocal of the absolute temperature (T). The slope between them gives an activation energy. Survival curves for cis-DDP (cisplatin) were exponential, although survival curves for bleomycin (BLM) were biphasic. Enhancement of cis-DDP and BLM effect was more substantial above 41°C than below 41°C. An arrhenius plot was made for resistant tails and a breaking point was observed at 41°C. The survival curves for 5-fluorouracil (5-FU) appeared to be biphasic, but the enhancement was small. The activation energy above 41°C was identical to the 2 other agents. No enhancement was observed for methotrexate (MTX). Unlike the other agents, no breaking point was observed for BCNU.

At Stanford University, Chinese hamster ovary cells (HA1) were exposed to therapeutic ultrasound in the presence of various drugs at temperatures ranging between 37 and 43°C. The survival of these cells was subsequently tested using the clonogenic assay. Marked enhancement by ultrasound of the cytotoxicity of adriamycin and amphotericin B was observed. For adriamycin, the potentiation was dependent upon the intensity of sonication. An increase in the intensity resulted in a decrease in the "threshold" temperature. The effect with adriamycin could be explained in part by an increase in net uptake of drug into the cells. Ultrasound was observed to increase the sensitivity of cells to adriamycin. The enhancement of amphotericin was only observed at exposure durations greater than 30 minutes and at 43°C. There was no enhancement observed for cisplatin and VP16. From these results, it appears that ultrasound

potentiates the cytotoxicity of drugs whose mode of action (at least in part) involves the plasma membrane.

Hyperthermia instrument development has been focused on systems that have the potential for heating tumors at depth in the body. At this time, the technology is such that ultrasound devices appear most likely to achieve this goal. At the University of Illinois, novel ultrasound phased array applicators have been designed via extensive computer simulation and implemented in prototype form. A 32-array prototype constructed on a section of spherical focusing shell, called the sector-vortex array, was implemented and shown to provide field patterns remarkably similar to theoretical numerically simulated predictions. A 64-element spherical section array prototype was also designed and tested with experimental results agreeing remarkably well with theory. Electronic systems and entirely new theory for computing the optimum phase distributions necessary to drive these arrays have also been designed and implemented. These new phased array applicators should give precise spatial and temporal control of energy deposition patterns for tumor heating at depth in the body.

Small business firms are also interested in developing hyperthermia systems capable of heating deep tumors. Labthermics Technologies, Inc. is developing a novel ultrasound frequency modification to electronically focus a three dimensional ultrasonic beam. Criteria for specifying the path and dwell time of the beam focal region to produce a uniformly heated volume will be defined, and a strategy for the placement of temperature monitoring probes to act as control points will be developed. Enhanced methods for therapy planning using various imaging and temperature probe insertions and localization techniques will be investigated to provide the therapy preparation methodologies necessary for practical clinical implementation of the system. The commercial potential of the proposed work is based upon the demonstrated effectiveness of hyperthermia for cancer therapy, and the present lack of sophisticated heating systems capable of three-dimensional control of a heating beam deep within the body. The proposed system is designed to heat deeply-seated tumors while sparing intervening and surrounding normal tissue. Such devices are not at present commercially available, though their need in the clinical environment is well known. Instrumentation is also being developed to improve temperature measurement and thermal mapping that is necessary during clinical hyperthermia treatments. The Luxtron Corporation is modifying and miniaturizing what is considered to be the best temperature sensor system commercially available. The capacity of the system will be increased three-fold from 8 to 24 channels. The new instrument will have the capability of monitoring the large number of probes that are needed for the treatment of large tumors by interstitial hyperthermia. A prototype of this instrument has been assembled and is being tested.

Thermal Technologies, Inc. is developing a device for accurate quantification of tissue thermal properties and perfusion (to below 5 ml/100gm. min to better than  $\pm 1$  ml/100gr.min). This device will open up a new physiological measurement capability in real time, repeatable or continuous, without toxicity. Motivated by its critical importance to hyperthermia therapy, tumor perfusion distribution, is central to determination of tumor eligibility for combined therapy. Tumor blood flow is the principal determinant of treatment temperature levels and uniformity and it is required for quantitative predictive temperature models and thus for thermal dose calculation. Further, the device will allow pursuit of important questions in tumor biology and in evaluating disorders or procedures with maldistributions of compromised levels of blood flow. Prototype instruments have been distributed to four hyperthermia centers evaluation.

Mathematic modeling of temperature distribution is being studied by many different investigators. At SRI International, the numerical model that was developed to compute thermal profiles by the finite difference technique based on both the Pennes (distributed heat sink) and the Weinbaum-Jiji (countercurrent heat exchange) bioheat equations, was used to design

perfused phantoms. Using these phantoms and thermography, the investigators are validating the numerical model which they developed.

The finite element method was also used at Wake Forest University to predict specific absorption rates in phantom tissues heated by external microwave applicators. When this method was combined with an optimization program to approximate the solution of the bioheat transfer equation, it was possible, at least in 2-dimensions, to calculate retrospectively the thermal distributions obtained during a clinical treatment. This work is extremely important for the interpretation of clinical hyperthermia data.

At Dartmouth College, a finite element time domain model for calculating SAR patterns in biological tissue was developed. This approach is said to allow for detailed representation of body geometry and tissue heterogeneity. Two dimensional work has been completed and preliminary 3-D calculations look promising.

A variety of clinical trials are being conducted or planned based on the new hyperthermia techniques being developed. At the University of Arizona, clinical trials have shown that their non-invasive scanned focused ultrasound system (SFUS) can selectively and successfully treat deep-seated tumors to higher temperatures than can be achieved by existing regional electromagnetic heating devices. Similar results were found for superficial tumors when compared to existing devices. At this same institution, other investigators are conducting a clinical trial using interstitial hyperthermia induced by inductively heated ferromagnetic implants. Both the University of California at San Francisco and City of Hope Medical Center will begin to participate in this study shortly.

At Purdue University, the preliminary work required to develop a closed-loop, interstitial, computer controlled hyperthermia system for intra-cranial neoplasms has been completed. Work has included software development, computational modeling, clinical data collection and analysis simulations of temperature distributions during interstitial heating of individual human intra-cranial tumors. The data obtained indicated that predicted tissue temperatures were typically within 1 degree centigrade of measured temperatures. The developed methodology will be used in the approved clinical trials soon to be initiated.

Ultrathermics, a small business grantee, is exploring a new approach and system for lung cancer hyperthermia based on propagating focused ultrasound (US) through lung tissue which has been temporarily filled with non-toxic liquids, termed "perfluorocarbons" (PFC). These liquids provide the acoustic propagation medium required to transmit the ultrasound. The procedure will be done in a manner which continually sustains patient respiration. In addition to improving lung cancer therapy, the techniques envisioned have the potential to benefit lung cancer diagnostic procedures. The commercialization of such a system, one with unprecedented efficacy and safety, is expected to find a wide and available market.

At the University of Cincinnati, a prospectively randomized study was designed to determine if hyperthermia (HT:  $44^{\circ} \pm 2^{\circ}\text{C}$  X 30 min X 1/week) increases the control of spontaneously arising tumors in veterinary patients (canine and feline) when combined with curative intent radiotherapy (RT: 3.5 Gy fractions X 14 given M-W-F) and how the interval between RT and subsequent HT affects tumor control and normal tissue tolerance. Analysis of evaluable cases from patients treated during the first two years of the study indicated increased complete tumor response (61% vs 44%) and a greater percentage of patients with long-term tumor control (64% vs 31%) with adjuvant hyperthermia. This enhancement appeared to be dependent upon tumor volume, histology and anatomic site. A four-fold increase in control was observed for tumors with volumes larger than  $10\text{cm}^3$  and 3-4 times more sarcomas were controlled by the combined therapy. In addition, enhancement of the acute skin reaction was also observed.

Longer follow-up intervals will be required to correlate late changes in normal tissues in response to these treatments.

### PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) is continuing to gain acceptance as a potential treatment modality for solid malignancies. PDT is based upon the principal that systemically administered photosensitizers appear to be preferentially retained longer by tumor tissue than normal tissue. When tumor tissue containing the photosensitizer is exposed to visible light with an absorption wavelength that is near the maximum for the photosensitizer, singlet oxygen is produced in the tumor cells. Cell death and tissue necrosis result. The increased interest in PDT as a mode of cancer therapy has stimulated the search for new photosensitizers and has promoted basic research on the cellular mechanisms associated with PDT.

Investigators are evaluating the feasibility of synthesizing and spectrally characterizing a large variety of long-wavelength cationic dyes as possible photosensitizers. Characterization will include *in vitro* and *in vivo* toxicity and pharmacokinetic studies. All living cells have a negatively charged electrical potential across their plasma and mitochondrial membranes which cause positively charged (cationic), membrane-permeable dyes to concentrate within cells and mitochondria. Many types of malignant cells accumulate higher concentrations of these dyes and retain them for much longer than do normal cells. One investigator has synthesized or obtained 40 dyes within four different chemical classes and evaluated them for selective photodamage *in vitro* and *in vivo*. The more promising compounds give selective killing ratios greater than 1000 between malignant and non-malignant cells in culture; a cell kill which compares favorably with antibody-targeted techniques. Using measurements of respiration in intact cells, these investigators have demonstrated that different cationic photosensitizers interact at different sites within the mitochondria and that many of the compounds can damage closed circular (e.g. mitochondrial) DNA, though the mechanisms of the photodamage appear to differ. Acceptable *in vivo* dose levels for 6 of their most promising cationic photosensitizers have been established and appropriate vehicles for their administration were devised.

The success of any drug therapy depends on getting the agent to the tumor tissue. Several investigators are looking at delivery systems relative to PDT. One investigator is trying to characterize and optimize selective light-induced killing of malignant cells using photosensitizers conjugated to monoclonal antibodies (MAB). Selective photodestruction of HPB-ALL human T leukemia cells using the sensitizer chlorin e<sub>6</sub> coupled via dextran molecules to an anti T cell MAB was achieved. Active conjugates directed against colon carcinoma and melanoma cells were also prepared. It was found that chlorin e<sub>6</sub> can be complexed with radioactive tin (<sup>113</sup>Sn), a gamma emitter which allows quantification of MAB-conjugate. Improved binding, imaging and therapy is anticipated.

The metabolic response of mammary adenocarcinoma in the C3H mouse to PDT is being investigated using *in vivo* <sup>31</sup>P nuclear magnetic resonance and microelectrode measurements. Animals were treated at subcurative and curative PDT levels. A distinct metabolic profile was identified for subcurative versus curative treatments. Forty-eight hours after PDT, all phosphate resonances were absent in spectra from animals subjected to curative treatments. In contrast, spectra from subcurative treatment, exhibited a transient decline in b-ATP followed by a return to near pretreatment intensities at 48 hours after treatment. Differences in spectral characteristics, distinguishing subcurative from curative PDT, were apparent as early as 4 hours post-treatment. In addition, a significant difference in tumor pH gradient (intracellular pH - extracellular pH) was detected 4 hours after PDT between subcurative

treatment and curative treatments. Thus metabolic markers have been tentatively identified which allow prediction of the biological outcome of PDT treatment of mammary carcinoma.

The effects of PDT in vivo on tumor metabolism, imaging, blood flow and perfusion was investigated in situ in real time using NMR spectroscopy. Using the recently developed spatially localized spectroscopy, a heterogeneous response to PDT that appears to be dependent on the depth of light penetration into the tumor, was demonstrated. In the outermost regions of the lesion, proximate to the irradiation source, the most dramatic and prolonged decline in ATP and increase in P was observed (1 to 3 hours after PDT). Furthermore, these same regions with time (24 to 48 hours after PDT) do not usually show a return of metabolites to pre-treatment levels, which sharply contrasts to the reversible depletion of ATP observed in the deeper regions of the tumor. Proton imaging demonstrates demarcation of necrosis in the outermost regions of tumors at 24 hours after PDT. Preliminary results suggest that blood flow is affected by PDT with about the same time-course as that seen for  $^{31}\text{P}$  metabolites. Additional studies are underway to define the causes, i.e., direct, cellular vs. indirect via vascularity, that lead to tumor cytotoxicity by PDT.

The effect of a new class of photosensitizers, i.e., purpurins on normal tissue was investigated. It was shown that 24 hours following administration, the reaction of normal tissue of light is minimal while at 48 hours after administration, no effect can be seen. Electron microscopic studies with purpurins indicate that when the photosensitizer is delivered via Cremophor emulsions, damage to endothelial cells precedes direct tumor cell damage.

Another investigator did a detailed analysis of photosensitizer content in tumor cells, light distribution in tumors and changes in tumor cell clonogenicity after in vitro or in vivo porphyrin-mediated photodynamic treatment which revealed a complex interplay between direct damage to tumor cells and secondary, tumor bed effects. The latter, expressed as disruption of the microvasculature, were found to be the major factors facilitating tumor destruction in transplantable, subcutaneous rodent tumor models. Rapidly developing tumor hypoxia during photodynamic light treatment can curtail the oxygen supply essential for the photodynamic process and thus limit treatment effectiveness.

The structure-activity relationships of new photosensitizers is being examined with the expectation that the findings will aid in the design of better photosensitizing agents. Early results have indicated that the very hydrophilic agents tend to bind to circulating high-density lipoprotein (HDL) and protein and sensitize at stromal loci or in lysosomes. More hydrophobic dyes bind to low-density lipoprotein (LDL) and HDL and sensitize at mitochondrial or membrane loci. Dyes which also sensitize endothelial cells in the vasculature appear to bind mainly to LDL. In related work, it was established that certain porphyrins and other dyes can also localize in atheromatous plaques, and that the resulting fluorescence emission spectra can yield information on plaque structure. There were distinct structural differences between plaque vs. tumor-localizing dyes.

Instrument development for PDT has been a dynamic area. Emphasis has been placed on improving the dosimetry and activating light sources for PDT. Fiber optic isotropic probes for measuring the space irradiance in vivo are being developed. Attempts are being made to increase the sensitivity of the fiber optic probes used for simultaneously measuring the fluorescence of the photosensitizing drug in vivo. A dosimetry monitor for measuring singlet oxygen emissions at 1270nm is being evaluated. In addition, several investigators are developing laser systems (diode laser and alexandrite laser) that will be compatible with the absorption wavelengths of the newer photosensitizers, many of which are in the 700-800nm range.

## RADIATION MODIFIERS: SENSITIZERS AND PROTECTORS

The pre-clinical and clinical research areas associated with radiosensitizers continue to be promising. The emphasis of the pre-clinical studies is on the factors that effect radiosensitization and the mechanisms involved. Clinical studies are evaluating the sensitizer SR-2508.

Several studies are investigating the basic mechanisms through which thiols operate to affect the sensitization of cells exposed to ionizing radiation in the presence of other important agents such as oxygen, or oxygen-like (electron-affinic) agents of the nitroimidazole class. One laboratory has reported the preferential radiosensitization of hypoxic cells to the level of oxygenated cells (i.e., the total elimination of the oxygen effect.) This was achieved in vitro with dimethylfumarate (DMF) acting on mammalian cells. It appears that the protein thiols, as well as the non-protein thiols, play an important role in this sensitization. Although DMF itself has limited solubility in vivo, it may well serve as a prototype for the development of compounds with similar chemical structures and/or properties which could be used in vivo without solubility limitations.

Other investigators studied the influence of cellular thiol content on the hypoxic cell cytotoxicity of radiosensitizers. They found that SR 2508 increased the rate of glutathione (GSH) depletion by buthionine sulfoximine (BSO), and SR 2508 was extremely toxic to hypoxic cells with lower GSH content. The major impact is on hypoxic cells with minimal effect on aerobic cells. These results encourage the use of BSO and SR 2508 in combination for clinical evaluation.

The problem of hypoxic tumor cells in radiation therapy is well known. Several studies have addressed this problem. The new bioreductive drug SR-4233, a benzotriazine di N-oxide, was shown to be highly effective in killing hypoxic cells in vitro and in vivo. It can also radiosensitize aerobic cells after hypoxic activation. This effect can be used to radiosensitize both aerobic and hypoxic cells in highly fractionated regimes (similar to those used in radiotherapy) under conditions in which classic hypoxic cell radiosensitizers do not work because of reoxygenation. Physiological means of altering tumor oxygenation in order to improve cancer therapy have also been investigated. The calcium channel antagonist flunarazine, at clinically usable levels, was shown to produce excellent radiosensitization in two different tumor types. This finding was similar to that previously reported with nicotinamide. Radiosensitivity appears to be correlated with improvements in tumor blood flow and oxygenation. Other vasoactive agents produce more modest effects. Investigators anticipate using both nicotinamide and flunarazine in pilot clinical studies shortly.

At the British Columbia Cancer Research Center significant progress has been made in the development of new fluorescent stains which will quantify tumor hypoxia at the level of the individual cell. Two new fluorescent probes have been identified and characterized in tumor models (spheroids) and mouse tumors. One of these stains has provided a new method to quantify transient changes in tumor blood flow. These investigators have also developed a simple new method for identifying and removing host cells from tumors, rapidly quantifying tumor cell respiration rate using fluorescent dyes and determining the role of transient changes in tumor hypoxia on binding of hypoxia markers. They also suggested a novel way to measure intracellular temperature using fluorescent dyes whose metabolism and binding is influenced by temperature. This observation could prove of importance to the field of hyperthermia where treatment outcome is dependent upon adequate heating all of the tumor cells.

The factors effecting radiosensitization and the mechanisms of radiosensitization have been addressed in several studies. Researchers at SRI International have studied the heterogeneous microenvironments and cells in tumor microregions. A significant correlation was found between tumor oxyhemoglobin (HbO<sub>2</sub>) saturation (measured by cryospectrophotometry) and tumor pH (measured by <sup>31</sup>P NMR spectroscopy). Inversed relationship between HbO<sub>2</sub> saturation and tumor volume or hypoxic fraction (assessed by cell survival curves) was demonstrated only in three (out of four) of tumor cell lines examined. Glucose diffusivity was quantified for spheroids of EMT6/Ro cells and five human cancer cells. These values suggested that a significant gradient in glucose concentration may exist in spheroids and tumors and glucose may play a role in the growth and cellular heterogeneity of spheroids and tumor. Greater hypoxic toxicity of misonidazole on wide type of CHO cells than glucose-6-phosphate dehydrogenase deficient mutant indicates other metabolic pathways in addition to hexose monophosphate pathway are also important in providing reducing equivalents for misonidazole activation. Experiments were carried out to determine the importance of growth factors, extracellular matrix and cell-cell interaction on radiosensitivity of human tumor cell. Almost in all cases, the radiation sensitivity of six human ovarian carcinoma cell lines were not modified by these three factors, therefore indicating that the *in vitro* predictive assay for clinical responsiveness of ovarian carcinoma would probably not vary significantly among these three factors. Enhanced radiosensitivity was observed in a human squamous carcinoma cell line, CaSki, if epidermal growth factor was added during the clonogenic assay period. The EGF-induced radiosensitivity increase was not correlated to its effect on plating efficiency, cell growth, recovery from potentially lethal and sublethal damages.

The efficacy and toxicities of a new series of perfluorochemical emulsions (i.e., perfluorocytlobromides) was studied by investigators at Yale University. These compounds appear to have clinical potential as adjuncts to both brachytherapy and external beam radiotherapy. There is a continuing interest in radiosensitizers as chemosensitizers. Nitroimidazoles (i.e. misonidazole) have been shown to be potential sensitizers of certain chemotherapeutic agents. At SRI International, two general areas have been investigated. Combinations of misonidazole with drugs such as melphalan, cyclophosphamide or CCNU plus the vasoactive compound hydralazine increased tumor response by as much as a factor of 3, with no increase in bone marrow toxicity in rodents. In the other study, the relative aerobic and hypoxic cytotoxicities of five new alkylating aziridino 2-nitroimidazoles were investigated in rodent and human cell lines *in vitro*. Significant differences were found among the drugs within and between different lines. Three of the compounds showed promising chemopotentialization with 4-hydroxycyclophosphamide.

The Phase III clinical trial of the radiosensitizer SR 2508, which is sponsored jointly the the NCI and Roberts Laboratories, is continuing to accrue patients. Patient accrual to this protocol is projected to be complete in 1989. Two other protocols using SR 2508 are ongoing at the Harvard Joint Center for Radiation Therapy. A Phase I continuous infusion study is nearing completion with responses being observed in breast, cervix and brain tumors. The clinical responses observed in the Phase II trial of prostate appear promising and other studies using SR 2408 are under development.

The work currently supported by the NCI in the area of radioprotectors is concerned with elucidating the mechanism(s) of radioprotection. At Massachusetts General Hospital, the focus has been on understanding thiol toxicity. These investigators have shown that manipulations of enzymatic pathways for detoxification of oxygen radicals can influence toxicity of added thiols. It has been postulated that thiols are toxic because of the production of hydrogen peroxide during thiol autoxidation, but these studies on eight different thiols show there is no simple correlation between thiol autoxidation rate and toxicity. However, these studies show that copper (II) is important for both thiol oxidation and toxicity. These results are of practical relevance for use

in establishing proper conditions for radioprotection studies with thiols and also are relevant to the current interest in oxygen radicals as damaging agents in chemotherapy, carcinogenesis, aging and arthritis.

Researchers at the Argonne National Laboratory have demonstrated that the inherent repair proficiency of cells is a major determinant in the level of radiation protection afforded by aminothiols compounds such as WR 2721 and WR 1065. Specifically, two repair-deficient CHO-EM9 and xrs-5 and proficient parental cell lines CHO-AA8 and K1 respectively, were studied. WR 1065 was effective in protecting AA8 and K1 cells against both radiation induced mutagenesis and cytotoxicity. In contrast, EM-9 cells which are defective in their ability to rejoin radiation induced-DNA single strand breaks were protected against cell killing but not mutation induction at the HGPRT locus. The cell line, xrs-5, which is defective in double-strand break repair was afforded no protection by WR 1065 against radiation induced cell killing but was protected against mutagenesis. These results suggest that it is the inherent repair proficiency of the cell which determines the extent of radioprotection by WR 1065 and not the ability of this compound to scavenge free radicals. They have also shown that WR-2721 exhibited anticarcinogenic properties, a finding that they will investigate further because of its potential use in young patients who are at risk of developing radiotherapy or chemotherapy induced secondary tumors.

Phosphorus-31 nuclear magnetic resonance spectroscopy is being used at the University of South Carolina for studying both in vitro and in vivo dephosphorylation of the radioprotective prodrug S-2-(3-aminopropylamino) ethylphosphorothioic acid (WR 2721, ethiofos) and its analogs. Experiments with a panel of rodent tumors and human tumor cells indicate that tumors vary considerably with respect to their alkaline phosphatase activity. Tumors that have significant levels of alkaline phosphatase may convert the prodrug WR 2721 to WR 1065, the active form of the drug. In such cases, WR 2721 will protect the tumor from the cytotoxic effects of radiotherapeutic and chemotherapeutic agents.

## RADIOLABELED ANTIBODY DIAGNOSIS AND THERAPY

Radiolabeled monoclonal and polyclonal antibodies and their fragments directed against tumor cell surface antigens have shown promise as both diagnostic and therapeutic agents in-vitro and in human tumor implants in animals. These observations have led to research in supporting this technology that is only now receiving significant federal funding. There are at least 4 foci of interest in this NCI research area; Radiation Research Program(RRP), Radiation Oncology Branch(ROB) and Biological Response Modifiers Program(BRMP) in Division of Cancer Treatment(DCT), and the Cancer Immunology Branch(CIB), Division of Cancer Biology and Detection(DCBD). Research is ongoing in the development of radionuclides for imaging and therapy. For imaging tumors, a low energy gamma emitter of about 150 KeV is optimum. For therapy a medium energy beta or high energy alpha emitter is necessary to deposit the energy to kill tumor cells. Investigations are studying the chemistry of linking the radionuclides and antibodies for greatest stability in in vivo.

Clinical trials are being carried out at certain research centers. The Radiation Therapy Oncology Group (RTOG) is conducting a Phase I/II study in the treatment of Hodgkin's disease with Yttrium-90 labeled antiferritin IgG. A Phase III trial of Antiferritin IgG labeled with Iodine-131 to treat malignant hepatoma is also being performed.

RDB is supporting of a Dosimetry Center, through RTOG, at Johns-Hopkins University. The Center is training other RTOG members in the computerized calculations of tumor volume



dosimetry. These dosimetric calculations will be useful in evaluating the effects of any type of cancer therapy on neoplastic masses.

As more is understood about the inhomogeneous distribution of radiolabeled antibodies, it is apparent more research is needed on dosimetry. Two grants received in response to an RFA on this topic were funded in FY 89.

A workshop on the Radiobiology of Radiolabeled Antibodies was conducted in the fall of 1988. Recommendations from this workshop are being developed with future RFA's and RFP's intended.

Strong central coordination of all of these research areas is necessary to thoroughly explore radiolabeled ligands/conjugates for the diagnosis and therapy of cancer.

### RADIOTHERAPY TREATMENT PLANNING

The Radiation Research Program continues to support a number Collaborative Working Groups made up of researchers from several institutions to investigate the many facets of radiation therapy treatment planning and treatment delivery. This continuing research effort emphasizes the three-dimensional (3D) nature of treatment planning and treatment delivery in the development of an optimal plan. One group evaluating three-dimensional photon beam treatment planning completed its contracts in 1988. Eight disease sites were investigated and the report summarizing the results and conclusions of the group are to be published in the fall 1989 as a supplement to the *International Journal of Radiation, Biology and Physics*. Another Working Group, charged with the development of guidelines and recommendations for interstitial brachytherapy, completed their work in 1989. The results of this three-year effort will be a reference textbook, to be published by Raven Press this fall. A third group is evaluating electron beam dose distributions using imaging techniques and computerized treatment planning systems. This research is scheduled to be completed in 1989, with the results to be published in 1990.

A new Working Group for Radiotherapy Treatment Planning Tools was established in the spring 1989, and has several tasks associated with bringing three-dimensional treatment planning into clinical practice. Currently, the process is extremely labor-intensive, and only a few institutions can accomplish it. The group will investigate computer-based software tools that allow contouring of normal structures and tumor volumes from a variety of medical images (CT, MRI, PET scans), displaying treatment plans and dose distributions in a three-dimensional environment; and monitoring on-line the patient's position during the actual treatment process. The group is made up of an interdisciplinary team of computer scientists, physicists and radiation therapists.

These investigators are breaking new ground in three-dimensional treatment planning, treatment plan optimization and evaluation of treatment delivery. Their scientific findings continue to be an important contribution to the planning and delivery of radiation therapy and, ultimately, to improved local tumor control.

### PATTERNS OF CARE

A contract to support a "Patterns of Care Study" (PCS) in radiation oncology was funded as part of an ongoing radiation research effort to determine the best current management (BCM) in the treatment of the five cancers: breast, cervix, Hodgkin's disease, prostate and recto-sigmoid. The PCS will survey the BCM of a representative sample of the U.S. radiation oncology facilities,

utilizing a facility survey. Additionally, a directory of all radiotherapy facilities in the United States and Puerto Rico will be generated. The outcome of the treatment of patients at those facilities will be studied in order that recommendations can be made to improve treatment, reduce complications, and increase patient survival.

## RADIATION BIOLOGY

The NCI, primarily through the Radiotherapy Development Branch ,RRP, DCT, continues to support a major portion of radiation biology research in the United States. Radiation Biology research is dedicated to improving radiation therapy as a treatment modality. Tumor and normal tissue radiobiology at the molecular, cellular and animal levels continues to be vigorously researched.

The following examples illustrate the breadth and diversity of this program.

- ( 1 ) Evidence is accumulating suggesting that thermotolerance is a state of enhanced repair rather than a state of reduced damage by heat. (This may provide a basis for diminishing thermotolerance, rendering hyperthermia a more effective treatment modality.)
- ( 2 ) There is evidence that boronated monoclonal antibodies designed to target cell surface tumor-associated antigens are not simply localized on cell membrane but are observed to penetrate membranes and reach both the cell nucleus and the nucleolus. (If this is true, it could have major impact on the efficacy of Boron Neutron Capture Therapy because of the relatively short range of the alpha particles involved.)
- ( 3 )  $^{31}\text{P}$  NMR spectral changes may be early indicators of response to radiotherapy for some tumors.
- ( 4 ) There is evidence that at least for head and neck cancer it is better to delay radiotherapy rather than to have delays during treatment. Furthermore, it appears the best therapeutic effect is obtained by using accelerated, hyperfractionated treatment where possible. Altered fractionation schedules are actively being studied by RTOG.
- ( 5 ) It has been shown *in vitro* that the cytotoxicity of adriamycin (ADR) was significantly enhanced by glutathione (GSH) depletion. The thiol depleting agent was buthionine sulfoxamine (BSO). The extent of ADR dose enhancement was found to be inversely proportional to the cellular GSH level at the time of ADR treatment.
- ( 6 ) Although quiescent cells have been known to exist in solid tumors, little is known about their radiobiology. Recent studies have demonstrated that human tumor quiescent cells are inherently more radiosensitive than proliferating cells. However, quiescent cells showed higher potentially lethal damage (PLD) repair.
- ( 7 ) It has been shown in dogs that spinal-cord evoked potentials were sensitive measures of radiation injury. Evoked potentials may be useful in humans to measure residual injury following irradiation to assist in making clinical judgements for re-irradiation or utilization of another modality which might be injurious to the spinal cord.
- ( 8 ) Research involving certain normal and radiosensitive mutant mammalian cell lines suggests that although recovery and repair processes play roles in determining cell survival, cell survival curve parameters may not always be useful in predicting cellular recovery capacity. Furthermore, there was no direct correlation between the abilities of the lines for

recovery following irradiation and their respective ability to rejoin single-strand or double-strand breaks. Thus, the relationship of these factors is much more complex than previously appreciated and will require alternative approaches for analysis.

#### SMALL BUSINESS INNOVATIVE RESEARCH (SBIR) GRANTS AND CONTRACTS

In FY 1989, the RDB funded 4 SBIR contracts (all Phase I) and 10 SBIR grants (3 Phase I and 7 Phase II). Funded research areas included photodynamic therapy, fast neutron therapy, boron neutron capture therapy, photon therapy, electromagnetic and ultrasonic hyperthermia, expert systems for Radiation Oncology and real time portal scanning for Radiation Oncology.

#### WORKSHOPS

Radiation Biology of Radiolabeled Antibodies - October 6-7, 1988.

This workshop reviewed low-dose rate radiobiology which is central to this rapidly evolving treatment modality. Radiation dosimetry problems result from inhomogeneous spatial and temporal distribution of the Moab. It is anticipated that one or more Program Announcements may result.

Potential Clinical Gains By Using Superior Radiation Dose Distributions - April 27-29, 1989

This workshop studied a variety of methods to achieve superior dose distributions in radiation therapy. The modalities discussed included charged particle therapy, conformal and dynamic therapy with conventional linear accelerators and the so-called "gamma knife" units, which deliver Cobalt-60 therapy through a specially-designed head applicator.

The Workshop concluded with a number of recommendations to the Radiation Research Program:

- (1) NCI appoint a committee to assess the national requirements for proton medical facilities;
- (2) NCI sponsor additional workshops to assess specific tumor sites the extent to which treatment volume can be reduced by using superior dose distributions;
- (3) the NCI sponsor a workshop on the importance of the volume of non-target tissues in the treatment volume;
- (4) the NCI support research for improving dose distributions with conventional therapy.

Screening Of Compounds For Radiosensitizing Activity - May 11-12, 1989

This workshop was convened to determine the need for and feasibility of developing a large-scale screening program for the detection of radiation sensitizers. Perhaps 2000-3000 compounds could be screened. Fourteen invited speakers represented the key, internationally recognized investigators in this research field. The consensus of the participants was that the MTT type of assay was a viable approach: It was the recommendation of the participants that the screening program be initiated by an RFA for a National Cooperative Drug Discovery Group.

## Clinical Trial Photodynamic Therapy (PDT)- September 1989

This workshop is scheduled for mid-September 1989. Participants will include clinical and extramural basic researchers with experience in treating various disease entities with PDT, as well as intermural NCI staff. It will be the aim of the workshop to identify 1) those diseases most likely to respond to PDT, 2) clinical protocols, and 3) institutions with the personnel, equipment and expertise who could accrue and treat patients. It is anticipated that a clinical trial cooperative group will be formed to study PDT in humans.

## FUTURE DIRECTIONS (RDB)

The Radiotherapy Development Branch will continue to stimulate, develop and administer clinical research and basic science research in radiation biology, chemistry and physics and support the development of advanced computer-based tools that improve the treatment planning and delivery of radiation therapy. The particle radiation therapy program, using both charged and uncharged particles, will continue to be a high priority research area for the near future.

The use of hyperthermia continues to be encouraging but further research needs to be performed in the development of deep heating units and of non-invasive thermometry. Hyperthermia as an effective adjunct to radiation and chemotherapy needs to be confirmed by standardized, randomized clinical trials for specific disease processes and anatomic sites.

Photodynamic therapy (PDT) is less well developed than the traditional disciplines of radiation oncology but because of promise in this area further research efforts will be stimulated. The present chemical compounds used for light-stimulated radiation treatments probably are not the optimal drugs for PDT. Newer photosensitizing drugs will require clinical testing.

Further research and development of radiolabeled immunoconjugates and cell specific receptors, a form of systemic radiation therapy (SRT), is necessary to explore the possibility of cellular radiotherapy. The dosimetry of these radionuclide tagged compounds is an important research area of this rapidly developing therapeutic approach and requires further support. Radiolabeled immunoconjugates for therapy and diagnosis will continue to be a high priority research area of the Radiation Research Program.

As research advances unfold in the chemistry and biology of boron containing compounds which preferentially concentrate in tumors, the interest in boron neutron capture therapy (BNCT) should increase. Irradiation of a boron compound with low energy neutrons causes emission of a short range alpha particle, which deposits an intense radiation dose at the cellular level. This is an additional example of possible cellular radiotherapy. The RDB anticipates an increasing role in this research area.

Radiosensitizers have demonstrated an ability to increase the sensitivity of neoplastic tissue to radiation and attempts will continue to improve the efficacy of these agents and to decrease their toxicity. New compounds need to be developed. This development is dependent on the capability to screen a large number of compounds for radiosensitizing activity. A more rapid screening system with greater capacity is needed if the area of radiosensitizers is to progress.

Research supported by NCI over the last decade has shown that the development of sophisticated computer-based treatment planning tools is essential to the routine use of three-dimensional planning and treatment in the clinic. New computer tools are needed that 1) support the management of medical images through computer networks, 2) develop decision-support aids for the radiotherapist in the therapy selection, tumor definition and treatment planning

processes; and 3) interface the computerized medical record with intelligent databases. Radiotherapy is the most computer-intensive discipline in medicine, primarily because of the anatomic information required to define the tumor and treatment volume, and the calculations that are needed to characterize the radiation dose to the tumor and the normal tissues at risk. Development of new computer-based systems that support the physician in all aspects of radiation therapy planning and delivery will result in better care and management for the cancer patient.

Dynamic conformal radiotherapy using conventional photon and/or electron beam accelerators is a new and exciting research area in which complex treatment plans are developed and implemented that conform precisely to the tumor treatment region, resulting in greater sparing of normal tissues. These developments will require advances in three-dimensional treatment planning, robotic vision techniques, expert knowledge systems and digital imaging verification systems. Technology transfer from the artificial intelligence community and medical informatics will greatly assist in this effort.

Medical informatics is a new and growing science concerned with the development of knowledge-based systems that are used to develop decision support tools, data management systems and physician workstation environments that increase the efficiency and personal productivity of the radiotherapist. The Patient Data Query (PDQ) system developed and supported by the NCI is a first step in creating systems that can take advantage of new technological applications that are available through the use of medical informatics techniques. Decision systems are needed to 1) support physician evaluation in selecting optimal therapy; 2) to follow patients placed on protocol to assure that clinical trials are conducted in an efficient and cost-effective manner; 3) to provide connections to clinical databases and laboratory test results used in the patient evaluation and diagnostic processes; and 4) tutorial expert systems for resident teaching and for continuing medical education in radiation oncology.

## PUBLICATIONS

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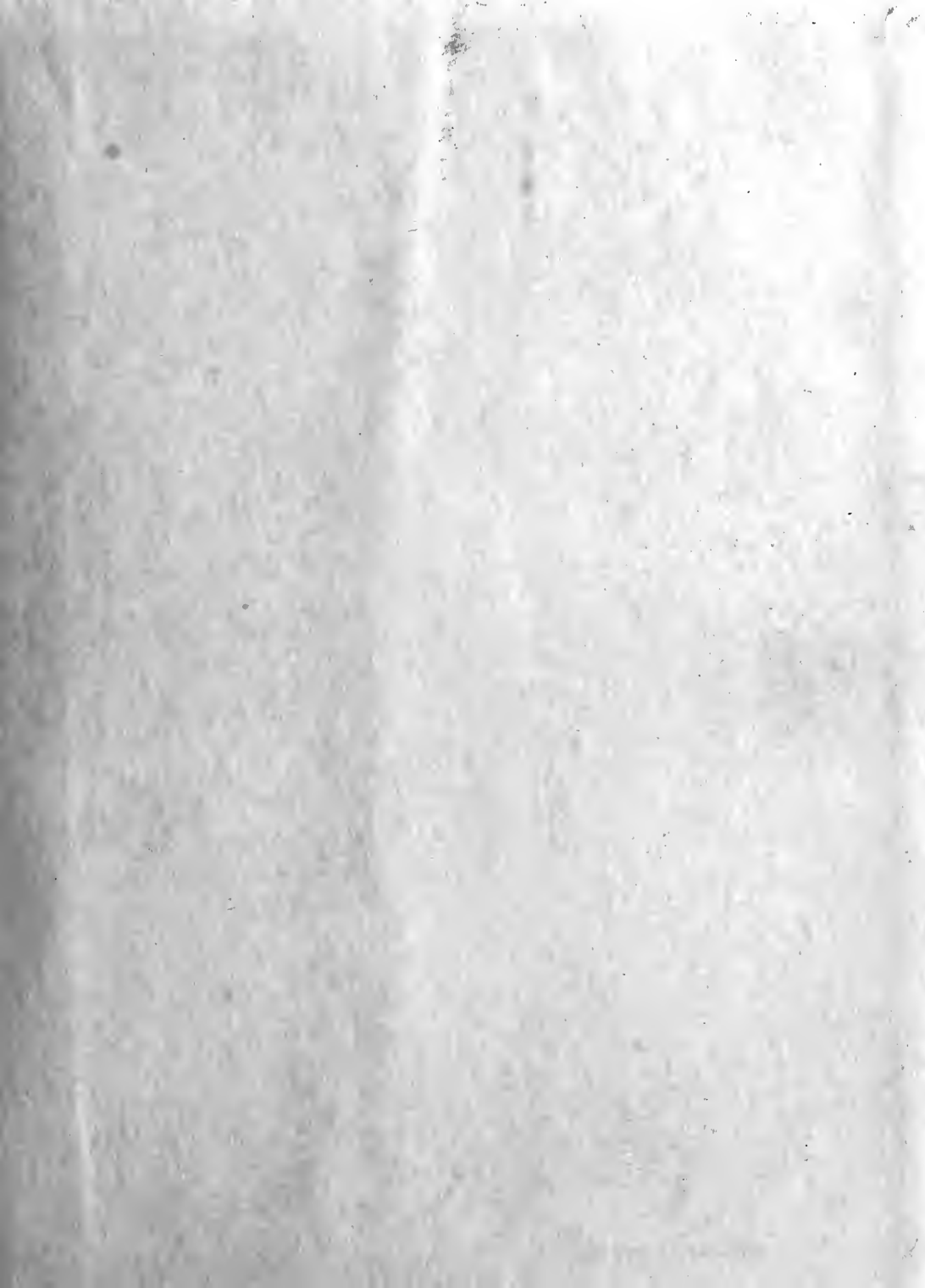
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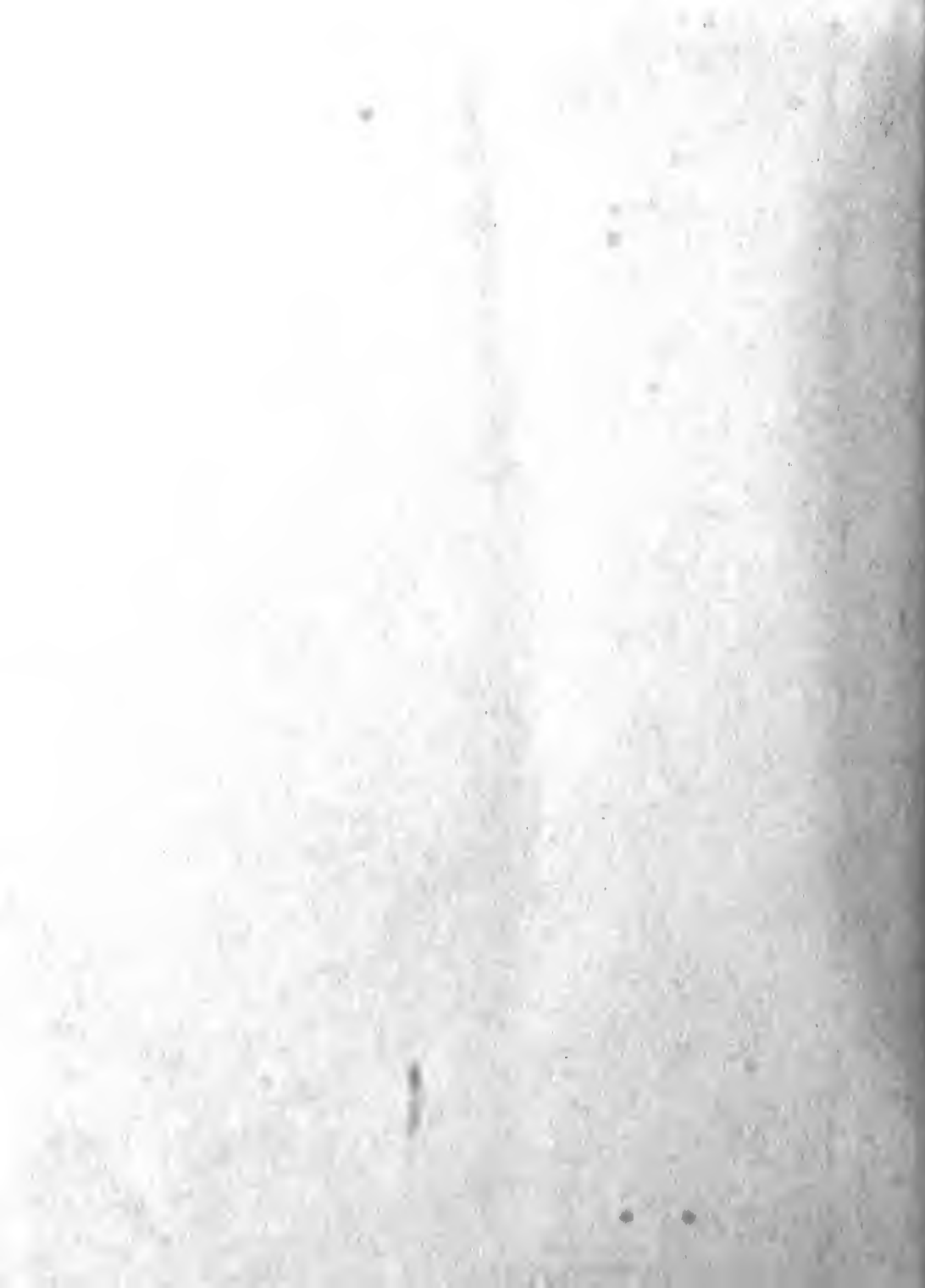




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